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VIRUS DISEASES OF PLANTS

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BY

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PREFACE

THE need for a small book upon the subject of Plant Virus Diseases appeared during my teaching work at Leeds University and in the County of Yorkshire. The study of this subject is, indeed, developing rapidly, but it seemed that this was not sufficient justification for withholding a simple text-book from the student of Mycology or Plant Pathology. A comprehensive volume would be inappropriate, as yet, and would have a limited use. The aim of this book has therefore been to introduce the student to the phenomena associated with Virus Diseases, rather than to provide him with descriptions of all known viroses; it is typical rather than complete.

Many pitfalls await the new worker on Virus Diseases, so it was decided to include descriptions of the various items of technique in common use. The list of references at the end of the volume will provide an introduction to the very extensive literature on the subject of Plant Viruses. In its compilation, accessibility and comprehensiveness of the papers quoted have been constantly in mind. The references cited in the text follow the general plan of the book, being representative, not exhaustive.

It is with great pleasure that I acknowledge my indebtedness to many persons—to my wife, for much constructive criticism and help, and to Dr. J. Henderson Smith, of Rothamsted, who criticized the manuscript in its early stages. Dr. J. Johnson, of Wisconsin University, U.S.A., has helped in ways too numerous to mention, and Prof. J. H. Priestley, of Leeds University, introduced me to the study of Virus Diseases of Plants, and helped considerably in its prosecution. Many workers in Ireland, Holland, America, Germany, and our own country have given written and verbal communication of their results. The Oxford University Press has throughout been most courteous and helpful.

June, 1933. J. G.

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Photographs used in Plates I–V and VI *a* by J. Manby, VI *b* by G. A. Nelson.

I

INTRODUCTORY

VIRUS diseases of plants have, during recent years, attracted much attention. This is scarcely surprising, for large losses on many crops are sustained annually as a result of their attack. These maladies do not always produce the same effects upon their hosts. Leaves may be mottled with light-coloured patches or yellow areas. The plant may be stunted or overgrown. The leaves may stand up almost vertically from the stem instead of assuming the horizontal position. Bunches of leaves may appear where normally they are spread thinly along the branch. Leaf blades may be rolled and may be chlorotic all over, whilst their veins often show red in colour. There is such a wide variation in the types of symptoms which may be produced that external appearances are not always reliable in the diagnosis of a virus disease.

Many research workers are studying these maladies, and it is now possible to define some of the properties of the causal agent. A virus is recognized as an ultra-microscopic particle which is probably smaller than the limit of resolution of the highest powered microscope. It is capable of passing through filters of diatomaceous earth or unglazed porcelain which would not allow the passage of bacteria. A bacterium may be about one twenty-five thousandth of an inch in length, whereas a virus particle is probably in the region of a millionth of an inch across—forty times smaller. A virus is infectious and multiplies in its host, where it does not exceed a standard maximum concentration. It is often very difficult or even impossible to determine the filterability of a plant virus, or to obtain any direct evidence as to its ultramicroscopic nature, so that most of these maladies are included as virus diseases

for the reasons that they are transmissible from plant to plant and that no microscopically visible parasite is demonstrable. All known viruses are parasitic and have not yet been cultivated in the absence of living tissue. Research workers are not yet unanimous as to whether the causal agent is living, or non-living, or even intermediate between these two states of matter.

Historical

As far back as the year 1757, a farmer called Maxwell described 'degeneration' of potatoes and stated that seed grown year after year in a lowland area gradually fell off in yield. He affirmed that the stocks needed changing to fresh ground or to a different country. Subsequent writers said that a stock of seed could be 'regenerated' by growth in a moorland district for a year or two. In 1764 it was recognized that a disease which curled the leaves was associated with degeneration, but the idea that continued vegetative propagation was responsible for the reduction in vigour had obtained a firm hold and ousted the disease hypothesis. It is now recognized that degeneration is caused by a progressive infection by virus diseases, and several stocks of potatoes which had wellnigh gone out of cultivation have been regenerated by the careful elimination of these maladies (80). Thus diseases which are now known to be caused by viruses were recognized more than a century and a half ago, but the true nature of their cause was unsuspected (15 and 81).

The beginning of conscious knowledge of virus diseases of plants dates back only to the beginning of the present century, when in 1899, Beijerinck, the great Dutch bacteriologist, was rather puzzled by a leaf-mottling disease of tobacco. He failed to find any bacteria associated with the malady, and was much astonished to find that a liquid which collected after juice from a diseased plant

had been passed through a bacterial filter, was still capable of reproducing disease when inoculated on to a healthy tobacco plant. The filter would not allow bacteria to pass, so Beijerinck argued that since the filtrate was clear, the cause of the malady must be an infectious liquid which multiplied within its host plant. He spoke of the disease agent as a 'Contagium vivum fluidum'—a living, infectious liquid (23). Mayer had demonstrated, many years before, that the causal agent of the mottling disease of tobacco was infectious (233), but it was Beijerinck who first turned our knowledge in the right direction.

Not long after Beijerinck's experiment, Iwanowsky made a detailed study of the same disease (184). He investigated the microscopic appearance of diseased leaves and described curious vacuolate bodies, which were later known as 'X-bodies', in the cytoplasm of the cells. His most important contribution, however, was the discovery that the virus was particulate in nature, and not fluid. This he found by comparing the diffusion of liquids, Indian ink, and virus filtrate into agar gels (p. 32).

The work of Allard (2–8) must take its place amongst the history of the study of plant viruses, for it first introduced us to such properties of the virus as heat inactivation, concentration, and longevity. Many interesting discoveries have since taken place and are portrayed in the following pages.

II

THE RELATION OF A VIRUS TO ITS HOST PLANT

Symptoms

There are, generally speaking, three types of virus disease symptoms—'Mosaic', 'Yellows', and a miscellaneous group. Fundamental differences of response in the host plant separate the first two classes. Mosaic diseases are readily transmitted by artificial and biological methods. Yellows diseases are spread by grafting and insects only (i.e. by biological methods; see p. 71). The imperfect state of our knowledge of the classification of virus diseases does not allow an exact subdivision of all maladies of this class into these three groups. The following lists will, however, show several diseases which are typical of each class.

Mosaic Diseases. Symptoms—mottling of leaves, with or without malformation. Transmission by mechanical and biological methods.

Tobacco Mosaic.	Bean or Legume Mosaic.
Yellow Tobacco Mosaic.	Potato Crinkle and Mosaic.
Cucumber Mosaic.	Dock Mosaic.

Yellows Diseases. Symptoms—general chlorosis, with clustering or upstanding habit of the young leaves. Transmissible by biological means only.

Aster Yellows.	Banana Bunchy Top.
Peach Yellows.	Potato Leaf-roll.
Strawberry Yellows.	Curly-top of Sugar Beet.

Miscellaneous. Symptoms various, neither mosaic nor

yellows. Transmission by either mechanical or biological methods, or both.

'Alloiophyllie' of Anemone.	Little Peach.
Witches' Broom of Potato.	Cranberry False Blossom.
Wheat Rosette.	Spindle Tuber of Potato.

External Symptoms of Mosaic Diseases.

Type: Ordinary Tobacco Mosaic. (Plate I.)

A tobacco plant infected with the mosaic virus is markedly smaller in all directions than is a healthy plant of the same age, and grown under the same conditions. Its leaves may be malformed by the slow growth of tissue round the vein tips, or along the whole or part of one side of the midrib. Lobing of the leaf margins and interveinal puckering often result from this unequal growth. There are frequently only two such areas, one on each side of the leaf apex, forming a mucronate point. The leaf blade is, in severe cases, reduced to a width of half an inch, whilst retaining a length of 4 or 5 inches. Complete suppression of the lamina, leaving only the midrib, is not rare.

The leaf blade itself is covered with areas which are coloured different shades of green. This mottling is always noticeable on the youngest leaves, and usually on the old ones also. The mosaic pattern can be demonstrated more clearly by placing a piece of white paper about an inch below the leaf, when some reflected light is transmitted through the lamina. The darkest coloured spots are often raised or slightly blistered above the normal leaf surface, and are darker green than a healthy plant. The lighter coloured parts are chlorotic, being very pale yellowish-green or almost white-green.

The blossoms of infected plants usually show a mottling of the pink areas of the corolla comparable to the mottling of the leaves, but it is not rare to find that flowers are

completely deprived of colour as a result of the disease. The flowers of species of *Nicotiana* whose corollas are normally white, greenish-yellow, yellow, purple, or red, are not speckled as a result of infection by Ordinary Mosaic virus. When a tobacco plant is infected early in its period of growth, it is not uncommon to find flower buds which are bent and dwarfed, and whose corollas are much shorter than the developing stamens.

The virus does not pass from the parent plant to the embryo, although it is present in considerable quantities in the immature fruit, the ovary wall, and other floral organs. It persists for several years in the dried chaff (calyces, petals, ovary walls, &c.) mixed with tobacco seed.

The roots of affected plants appear to be quite normal.

Symptoms of Yellows Diseases.

Type: Potato Leaf-roll. (Plate VI.)

The primary symptoms are a slight inward rolling of the margins of the lowest leaves together with a general stunting of the whole plant. Later, the young leaves become affected, and remain standing erect round the growing point, instead of assuming the normal horizontal position. Individual leaflets are quite yellow, and are rolled in a funnel-shaped manner. The main veins on the back of each leaf are usually red. Flowering tends to occur prematurely, though the plant is usually severely dwarfed. When the whole plant is infected, the disease is called 'secondary' Leaf-roll.

The Spike disease of Sandal (*Santalum album* L.), which also belongs to the Yellows group, causes the leaves to become yellowish and shortens the axis which bears them, thus producing a bunch of leaves at the apex of a shoot. Aster Yellows produces no rolling of the leaves or

reddening of the veins of infected aster leaves, but otherwise produces symptoms generally similar to those of Leaf-roll. (See also p. 60.)

Miscellaneous Symptoms. 'Alloiophyllie' of Anemone causes the leaves to be malformed. The lobes are not formed symmetrically, and frequently a leaf may be bent right round until its apex reaches the petiole. Witches' Broom causes a multiplicity of weak, spindly shoots to arise where normally one or two strong stems would appear. There is usually a reduction in the size of leaves borne by diseased stems. Wheat Rosette causes the plant to tiller extensively and remain vegetative. Little Peach disease produces diminutive fruits which ripen later than normal, whilst the leaves are small, yellow, and clustered. A flower which becomes vegetative, and sends out an erect shoot, instead of becoming a pendulous fruit, is typical of Cranberry False Blossom, whilst Spindle Tuber, as its name implies, produces potatoes which are much elongated.

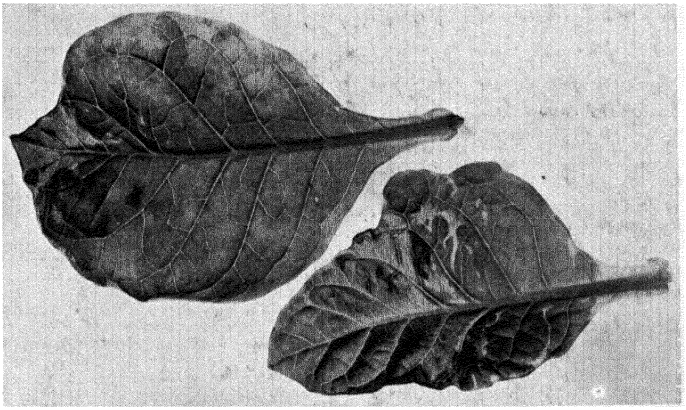
Symptom Complexes: The Potato Virus Group. Potato Streak has long been known as a disease induced by more than one virus acting in the same host, and it is known that when a tomato is infected with Tobacco Mosaic and with the virus carried by American potatoes (p. 8), it develops a very severe streak which is usually lethal (140). Dr. K. M. Smith found that different symptoms were produced when potato Crinkle was transmitted by needle inoculation from those produced when aphids spread the disease (370). This suggested that Crinkle was caused by more than one virus, and other workers have also proved this to be the case (271, 327, 328, 330, 373; cf. also 312). There appear to be no less than five distinct viruses (377): three designated by letters only—'A' (271), 'X', 'Y' (373), 'Up-to-Date

virus' (271) and 'Para-crinkle' (330). The symptoms formerly known as 'Interveinal Mosaic', 'Crinkle', and 'Streak' are combinations of two or more of these separate viruses, which all behave differently in regard to their transmission, host-range, and symptoms on different hosts.

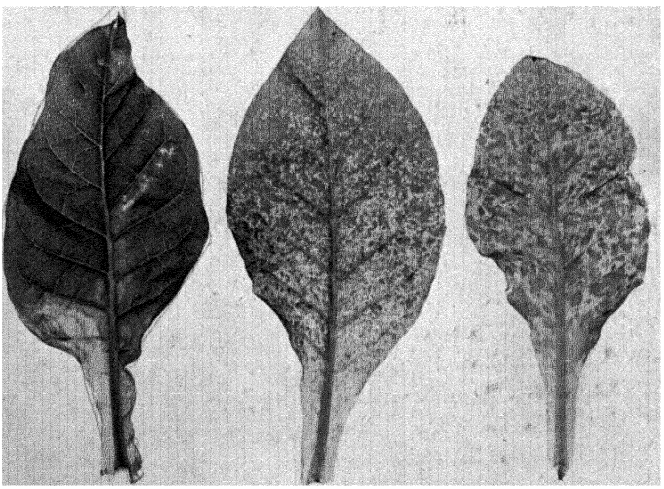
The Host Range of Virus Diseases. The virus of Tobacco Mosaic attacks many hosts (p. 57), all of which belong to the Natural Order Solanaceae. Tobacco Ringspot has an even wider range, whilst Aster Yellows has been transferred to plants belonging to twenty-three Natural Orders (p. 60). Symptoms upon the different hosts are not all the same. The virus of Tobacco Mosaic, for instance, produces a severe mottle upon the leaves of *Nicotiana tabacum*, but a very severe browning or necrosis on the leaves of *N. rustica*. Plants of the latter species are, indeed, frequently killed by the disease. It is therefore necessary, when describing symptoms, to state the host on which they are observed.

Carriers. 'Carriers' are plants which allow the multiplication of virus within their tissues, but show no symptoms. *Solanum pyracanthum*, for instance, is a carrier of the Ordinary Mosaic of tobacco. It shows no mottling or malformation after inoculation, and gives no symptoms whatever; but if its leaves are ground in a mortar about a fortnight after infection, and the juice inoculated to healthy tobacco plants, the latter will ultimately show symptoms of typical Tobacco Mosaic. There is some indication that the properties of the virus (Chapter III) are altered slightly when it is in the carrier variety. Its heat-inactivation point is lower, and it does not reach the same maximum concentration in its host.

The potato variety British Queen, in common with most American varieties, harbours a virus whose presence can



Leaves from a tobacco plant infected with ordinary mosaic



Leaves from a tobacco plant infected with yellow mosaic, to show different types of mottling

be demonstrated by inoculation on to tobacco, when a slight, dappled mottling is produced. (Plate V.)

Increase and Decrease of Virulence. Subsequent transfers on tobacco of the virus mentioned in the last paragraph produce symptoms which become more severe with each successive transfer. After eight or nine transfers, the virus gives either a very severe mottle or a series of necrotic spots. This seems to be a case of increase in virulence of the virus by passage through a particular host (194). A similar phenomenon has been reported for the curly top virus of sugar beet, which is attenuated by passage through carrier plants of sugar beet, through *Chenopodium murale*, *Rumex crispus*, and *Suaeda moquini* (60), and restored to its original virulence by inoculation to *Stellaria media* (217, 218).

Resistant Varieties. All varieties of a species are not equally susceptible to attack by virus diseases. For instance, many varieties of cucumber are resistant to Mosaic (301), i.e. they show very mild symptoms of the disease. The new POJ varieties of sugar cane are highly resistant to Sugar Cane Mosaic (387). Many plants of sugar beet are resistant to Curly Top disease.

Immunity. It will be obvious that since the host range of a virus is restricted, there must be plants which do not allow the virus to enter their tissues. In some instances, this immunity becomes intraspecific—i.e. within a species there are varieties which are susceptible, and others which are immune. Thus some bean varieties are immune to Mosaic, though most are susceptible. The species of sugar cane, *Saccharum spontaneum*, is immune to Sugar Cane Mosaic. There seems to be no proof of *acquired* immunity to virus diseases (109, 303, 377).

Recovery. Several instances have been reported where infected, susceptible plants have apparently made symptomless new growth at normal temperatures (51, 109 *et al.*). The virus causing Maize Streak apparently attacks certain varieties of sugar cane (e.g. Uba), producing mild symptoms which rapidly disappear. Subsequent growth does not appear to contain the virus (396).

The Masking of Symptoms. The activity of many viruses seems to be greatest at temperatures below those at which the respective host plants flourish most. This means that by growing the plants at a high temperature, the young leaves as they appear will show no symptoms, or only exhibit them in a mild form. Dr. J. Johnson found that when tobacco plants infected with Ordinary Mosaic are grown at a temperature above 37° C., the new leaves produced under these conditions have none or only very slight symptoms (193). The appearance of the symptoms is 'masked', but the virus is present in the leaves, as can be proved by re-inoculation of their tissues to healthy plants. Potato plants infected with Crinkle show no symptoms on leaves produced when the temperature rises above 75° F., and a temperature of this order for five hours per day is sufficient to cause masking (406). If both the host plants mentioned are returned to cooler temperatures, symptoms will remain mild on subsequent growth of tobacco, whilst the potato will develop new leaves showing characteristic Crinkle symptoms. The older leaves of the plant, matured and diseased before exposure to high temperatures, do not lose their symptoms as a result of this treatment.

Other virus diseases exhibit masking, but the Ordinary Tobacco Mosaic stands by itself, as yet, in that a permanent attenuation is given by growth at high temperatures. The phenomenon of transient masking is perhaps explicable by the supposition that the virus has an optimum temperature

of activity lower than that of its host. The practical significance of masking is discussed under Chapter V (193, 195, 381, 406, 437).

The Effect of Shading upon the Development of Symptoms. It has been established experimentally (358) that symptoms of Tomato Yellows are most severe when the host plant is grown in bright light, and less so in the shade. This has also been reported for Curly Top of Sugar Beet.

Transmission

Seed Transmission. Although most virus diseases are not transmitted by seed from a diseased plant, a considerable number find their way into the seeds, which then produce seedlings, of which a certain percentage may be diseased. Bean Mosaic (Plate II) is most readily transmitted by seed, but rarely produces more than 50 per cent. of diseased seedlings. The following list enumerates ten virus diseases which may be transmitted by the seed of one or more of their hosts:

Bean Mosaic	Leaf-roll of Potato	Aucuba Mosaic of Potato
Lettuce Mosaic	Cucumber Mosaic (through wild cucumber seed)	Tobacco Ringspot (through Petunia and Turkish tobacco seed)
Legume Mosaic	Beet Mosaic	Tomato Streak.
Tomato Mosaic		

Pollen Transmission. A report has recently been published (318) that the Mosaic of the Bean is transmissible by means of the pollen. Flowers of healthy plants were emasculated and fertilized with pollen from a diseased plant. Seeds produced from such a union gave seedlings, of which a considerable percentage were diseased.

Soil Transmission. Tobacco Mosaic, several virus diseases of the potato, and the Rosette Disease of Wheat appear to be transmitted through the soil. If diseased tobacco roots are present in soil which is used to raise seedlings of that crop, the decaying roots will provide a source of virus infection, since the seedlings are slightly wounded during the process of transplanting. Transmission of the potato viruses seems to be the work of the larval form of *Tipula paludosa*, which eats the roots of diseased plants and then passes to healthy ones (115, 200, 427).

Virus Diseases and Vegetative Propagation. It is now known that increasing infection with virus diseases is responsible for the 'degeneration' of stocks of many vegetatively-propagated plants. This was formerly attributed to continued vegetative multiplication, but many plants, of which the banana is perhaps the best instance, have always been propagated vegetatively, without any diminution of vigour since they were first introduced to commerce. Vegetative propagation, however, is particularly suitable for the spread of virus diseases, owing to the relatively systemic nature of the latter (p. 18). Seed propagation, as we have seen, often does not transmit virus, and when it does, only a low percentage of seedlings is infected. *All* the cuttings, tubers, scions, or divisions made from a diseased plant carry the virus. Thus we see that although vegetatively propagated races of plants do not degenerate as a result of lack of sexual fertilization, they nevertheless provide conditions for the very rapid and complete spread of virus diseases. This is well seen in the case of the potato, where the 'life' of a variety used to be about twenty years. We now know that this meant that the whole stocks of this variety had become so completely infected with virus diseases that their yield had fallen below that of economic production. It has already been mentioned

(p. 2) that such a stock could be 'regenerated' if all virus diseased plants were eliminated.

Insect transmission is discussed in Chapter IV, and mechanical methods and grafting are described in Chapter VII.

Histology and Cytology

Microscopic Anatomy of Diseased leaves. The dark green areas of a Mosaic-infected tobacco leaf, when examined microscopically in transverse section, are about one and a half times as thick as the yellow areas.[1] They may even be thicker than the normal healthy leaf, and frequently have two rows of palisade cells. The spongy parenchyma is usually normal in form, but may be either looser or more tightly packed as a result of virus infection. Cells of the palisade layer of a chlorotic area are cuboidal, or may be up to twice as long as broad when seen in transverse section. They have fewer plastids per cell than normal tissue, and those which are present are small and often partially disintegrated. The transition between green and chlorotic areas is very sharp.

Inclusion Bodies. Peculiar spheroidal bodies, which are apparently vacuolate, are often found near the nucleus of diseased cells. They are known as 'X-bodies' or 'Inclusion bodies', and are found only when certain diseases attack certain hosts. Most species of plants which are susceptible to Ordinary Tobacco Mosaic produce X-bodies, and they have been found in all tissues except the apical meristems and cambium of a tobacco plant infected with this malady. They occur in the seventh and deeper layers of cells of the growing point, in the leaf and branch primordia, and even in the phloem.

[1] An exception is found in Sugar Cane Mosaic, where the light green areas are the thicker.

Rectangular, translucent bodies often occur in cells of diseased plants. They are usually in association with the

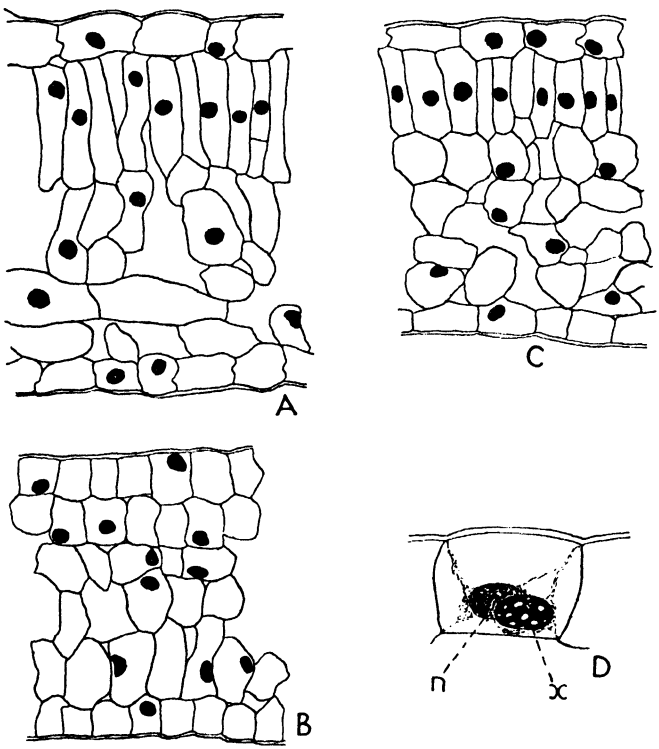


FIG. 1. A. Transverse section of a very dark green area of a Mosaic-diseased tobacco leaf. B. Transverse section of a light green area of the same leaf. C. Transverse section of a healthy tobacco leaf. D. An X-body (*x*) in close association with the nucleus (*n*) of an epidermal cell of a Mosaic-infected tobacco leaf. A, B, and C are magnified 200 times; D, 400 times.

X-bodies but are sometimes found independently, as in xylem vessels. Treatment with the usual fixing fluids causes them to show fine lines running parallel to the short length of the plate, and from this character they are called 'striate material'.

The mechanism by which X-bodies are formed has recently been investigated (361). The protoplasm in cells of *Solanum nodiflorum* diseased with Tobacco Mosaic was observed to stream. Places where the protoplasm was more dense seemed to amass the streaming substance round them, ultimately building up the characteristic body. The bodies frequently disappeared again by the erosion of the protoplasmic stream. Other workers have noticed that the bodies sometimes divide when the host cell divides, thereby spreading themselves through the plant tissue.

The general consensus of opinion amongst workers on virus diseases is that the bodies represent an abnormal product of the host cell as a result of attack by the virus. This seems to be supported by all the available evidence. The possibility of the inclusions being aggregates of virus particles is, however, not wholly eliminated.

The presence of X-bodies in cells may be demonstrated by staining microtome sections of the host tissue with iron-alum Haematoxylin, which produces a deep purple colour in the inclusions. Fleming's Triple stain is also useful. Inclusions may be seen in unstained hair cells, freshly mounted in water, or a piece of epidermal tissue may be similarly treated. A 1/12″ oil immersion objective will be needed on the microscope used for examination. The presence of X-bodies and striate material is not characteristic of all virus diseases. The inclusions may appear on all plants in the host range, but their occurrence is not sufficiently regular to be of diagnostic value.

Some Host-Virus Combinations which produce X-bodies.

Cabbage Mosaic.	Dahlia Mosaic.	Fiji Disease of Sugar Cane.
Hippeastrum Mosaic.	Petunia (Tobacco Mosaic).	Potato Mosaic.

Spike Disease of Sandal.	Stunt Disease of Rice.	Sugar Cane Mosaic.
Tobacco Mosaic.	Wheat Mosaic and Rosette.	Yellow Mosaic of Tobacco.

References: 72, 96, 134, 147, 148, 149, 168, 169 (Rev.), 174, 175, 210, 211, 249, 250, 317, 338, 361.

Local Lesions. It is usually found that the inoculated leaves of a plant develop no symptoms, but if inoculation is performed before the leaf is more than one centimetre long, the virus of Tobacco Mosaic produces a chlorotic mottle along the veins. If, however, the same virus is inoculated on leaves of *Nicotiana glutinosa* by pricking with a series of insect pins, or rubbing lightly with a cloth, numbers of yellow halo-like spots develop in the neighbourhood of the inoculation. These are caused by the virus, since pricking or rubbing with non-infectious pins or cloth will not produce them. The number of these lesions is proportional to the concentration of the virus. Similar lesions can be demonstrated if Turkish tobacco leaves are inoculated in a similar manner, and treated with iodine (179).

Necrosis. Some virus diseases cause certain parts of their hosts to become brown and shrivel. This is called 'Necrosis'. A necrotic condition of the phloem is very prevalent in potato plants affected with Leaf-roll—in fact, the disease used to be called 'Phloem Necrosis'. It is now recognized that Leaf-roll produces a large amount of phloem necrosis, but this condition is also found in phloem of healthy plants. Several other forms of necrosis of potato plants are described—'Acronecrosis' (= 'Top Necrosis'), 'Acropetal Necrosis', 'Pseudo Net Necrosis', and 'Concentric Necrosis' (14, 21, 135, 142, 143, 308, 329, 339).

Movement of the Virus in its Host

The Spread of the Virus in its Host Plant. An infinitesimal amount of virus is introduced when a plant is inoculated by the prick of an infection-bearing needle. Yet it ultimately fills nearly all parts of the host. This involves

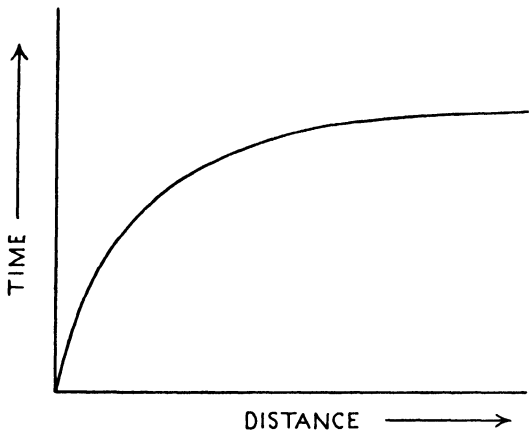


Fig. 2. Diagram showing the relation between time and distance in the spread of Ordinary Mosaic virus through tobacco.

spread as well as multiplication. The rate of this spread can be studied by inoculating a large number of plants at a particular place on each of them, and cutting off the leaf or stem at varying distances, after different intervals of time. The plant will make new growth which will show, by subsequent production or absence of symptoms, whether the virus had passed the distance between inoculation and severance in the given time. Fig. 2 shows the results of such an experiment on tobacco infected with Ordinary Mosaic (154).

The manner of spread portrayed by the logarithmic curve appears to hold for whichever direction the virus spreads—whether upwards or downwards in the stem or

leaf. The spread upwards is not markedly faster than the spread downwards. The rate varies, however, for different viruses upon different host plants. The Streak Disease of Maize, for instance, travels down the leaf at the rate of 30 cm. in 3 hours. The petiole of the sugar beet allows the virus of Curly Top to move at the rate of 7 inches in half an hour. These rates are too high for spread caused by diffusion, and some of them are too slow for mechanical carriage by the water-stream in the xylem (169).

The virus must be able to move about a tobacco plant from cell to cell of the parenchyma, for a plant can be infected by scratching the hairs on the leaf, or stem, by means of a razor which has been made to perform a similar function on a diseased plant. There may also be mechanical transport of the virus by the streams of water and nutrients in the vascular system. Some viruses, as Raspberry Mosaic, are apparently only transferred by the phloem of their host plant, since ringing of the stem to the xylem stops spread of the virus.

Symptoms do not appear on the young leaves immediately after inoculation. An 'Incubation period' must elapse. This is a function of the time taken by the virus to travel from the point of inoculation to the young leaves, and to produce its effects upon the developing cells.

The Concentration of the Virus in its Host Plant. Since the virus moves to almost all parts of an infected plant, it is said to be 'systemic'. It must be realized, however, that this is only relative, for there is at present no evidence that the virus can enter meristematic tissues. Moreover, the virus is not at the same concentration in all parts of the host—it is, for example, about ten times stronger in the light green than in the dark green parts of a mottled leaf (154, 177). When the virus spreads in the host, it first multiplies to a high concentration in the neighbourhood

of the inoculation, and moves at a lower concentration which later increases (154, 180).

Whilst the concentrations of different viruses on different host plants vary greatly, there is usually a standard maximum concentration which is not exceeded, and this

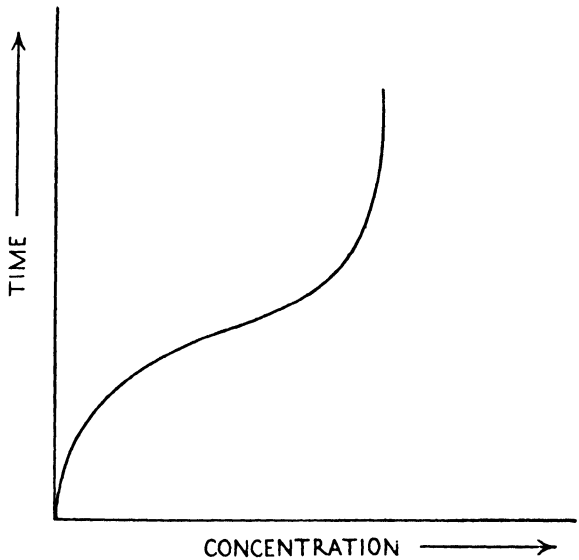


FIG. 3. Diagram showing the rate of multiplication of the Ordinary Tobacco Mosaic virus in its host plant.

is sufficiently constant to be of diagnostic importance. The virus of Ordinary Mosaic on tobacco is usually sufficiently concentrated to give infection when juice from diseased leaves is diluted with water to 1 in 100,000, but not to 1 in 1,000,000. Cucumber Mosaic on tobacco is infectious at a dilution of 1 in 100, but not at 1 in 1,000. (The method of estimating concentrations is described in Chapter III.) This standard maximum concentration apparently holds for all parts of the plant—leaves, stem, and roots—except the meristematic areas.

The virus reaches its maximum concentration at a logarithmic rate which is portrayed by Fig. 3 (154). This knowledge is obtained by cutting out strips of tissue from a leaf, as near as possible to the region of inoculation. The concentration of the virus in comparable strips is estimated after successive intervals of time. It is interesting to note that the S-curve of Fig. 3 portrays also the rate of accumulation of the catalyst in an autocatalytic reaction, and the rates of increase of a colony of bacteria in liquid culture, and of the growth of most plant organs.

Physiological Effects

The pattern of a tobacco leaf infected with Mosaic virus consists of a ground of normal green with patches of lighter and darker green. The patches usually show no relation to the veins, and are sometimes crowded towards one end of the leaf, sometimes towards the other. All conceivable patterns occur. The differences in colour are due to variations in the amounts of the photosynthetic pigments. The green pigments are usually present in greater proportions in the dark parts, and in smaller quantity in the light green areas, as compared with a healthy leaf. There is a large increase in the amount of Carotin in the dark green parts, but not quite such a big increase in the light green areas. The amount of Xanthophyll in both dark and light parts is reduced (114). Carbohydrates are always more abundant in the dark parts—in fact starch is usually absent from the light green, or chlorotic parts. Healthy leaves always contain more starch than diseased ones, taken as a whole. The *proportions* of hexoses, non-reducing sugars, and insoluble carbohydrates are the same in healthy as in diseased leaves, suggesting that the small amounts in the latter are not due to any dislocation of the mechanism of carbohydrate metabolism.

There are, however, some mosaic diseases like Spinach Blight, which are characterized by an accumulation of starch in diseased leaves (408). There would seem to be a good case for supposing that the virus of this disease causes considerable dislocation in the translocation mechanism.

When compared with healthy leaves, diseased leaves usually contain more total nitrogen and protein, variable amounts of amino-nitrogen, but similar amounts of other forms of nitrogen. This state of affairs seems to indicate a disturbance of normal nitrogen metabolism—probably a slower oxidation of amino-acids and protein break-down. This may be correlated with a greater carbohydrate oxidation and would account for the lower carbohydrate content in diseased leaves.

No constant variation can be claimed for the relative acidity (pH) of the sap of a mosaic-infected host from that of the corresponding healthy plant.

References: 28, 35, 48, 75, 78, 89, 106, 107, 108, 114, 118, 189, 190, 229, 293, 325, 363.

The Spike Disease of Sandal, a Yellows disease, causes a slight increase in total nitrogen, in water soluble nitrogen, and in amino-nitrogen, with decreases in nitrates, proteins, and polypeptides, as compared with healthy plants. Potato Leaf-roll interferes with the translocation of starch from the leaves, and since they become 'gorged' with this material, the leaf blade is often brittle to the touch, and rolled, hence the name. The interference with starch transfer is apparently not due to lack of the appropriate enzymes (403). Whilst tubers from Leaf-roll plants show a decrease in dry weight as compared with healthy, it is not infrequent to find that the leaves have an increase in dry weight. The 'starch test' is a good means of testing for Leaf-roll in its early stages. Leaves from suspected plants are gathered

along with healthy ones of the same variety, and both are placed with their cut ends in water, in the dark, for twenty-four hours. They are then killed with boiling water, extracted with alcohol, and tested for starch with iodine solution. The healthy leaves should show no black colour, whilst the diseased ones will show it abundantly, except, possibly, a little area round the base of each leaflet. This is noteworthy, since starch disappears first from the *tip* of a healthy leaf.

In Leaf-roll potato plants, photosynthesis is considerably reduced in the 'secondary' stage of the disease. Transformation of starch to hexose, hexose to sucrose, and sucrose to starch takes place in the leaves, but there is very little translocation. Carbohydrates are transported as hexose, as against sucrose in healthy leaves. The hexose appears to move slowly through the parenchyma of the petiole, and not in the phloem (19).

References: 19, 52, 53, 59, 251, 263, 273, 316, 336, 380, 383, 384, 403, 434. Also 377 (Rev.).

Developmental Anatomy

Infection by Mosaic diseases delays the rate of vacuolation of cells of a developing leaf primordium, as compared with a healthy leaf, but it also causes local irregularities. Groups of cells in a diseased leaf begin to vacuolate before their surrounding fellows. The anatomical features associated with a light green area (p. 13) would be formed if the future palisade cells reach complete vacuolation before the upper epidermis. The palisade cells will cease division sooner than the surrounding cells, and since there will be relatively few cells per unit volume, they will exert little mutual pressure, and tend to be as long as broad, and of equal depth. The cuboidal shape of the palisade cells of a light green area is thus explained. The anatomical characters associated with an area darker

green than normal are palisade cells very much elongated in a direction at right angles to the leaf surface, and their frequent disposition in two layers. This state of affairs would be brought about if the palisade cells were delayed in reaching complete vacuolation relative to the upper epidermal and the spongy parenchyma cells. They would remain in a dividing condition for a longer time, and the larger number of cells thus produced would give mutual pressure, which would cause the cells to elongate in the characteristic manner. If the process were delayed considerably, the elongated cells would begin to divide by walls across their smallest diameter, and would thus produce the two-layered palisade characteristic of a very dark green area. A normal sequence of vacuolation would produce a tissue having normal anatomical features.

Disintegration of chloroplasts has been observed in mature affected tomato plants, but there is also evidence to show that virus inhibits the formation of chloroplasts in early stages of development (156, 362, 378).

Organisms associated with Virus Diseases

Many Protozoa, Slime Fungi, and Bacteria have been reported in association with virus diseases (see the review 377), and have even been ascribed as causes. Such claims frequently ignore the main criterion of a virus—its ultramicroscopic nature. The probable role of such organisms is shown in a recent report (170). Organisms are found in diseased plants grown under normal conditions, but not if precautions are taken to make growth bacteriologically aseptic. The virus, however, produces symptoms under both conditions.

III

PROPERTIES OF THE VIRUS EXTRACT

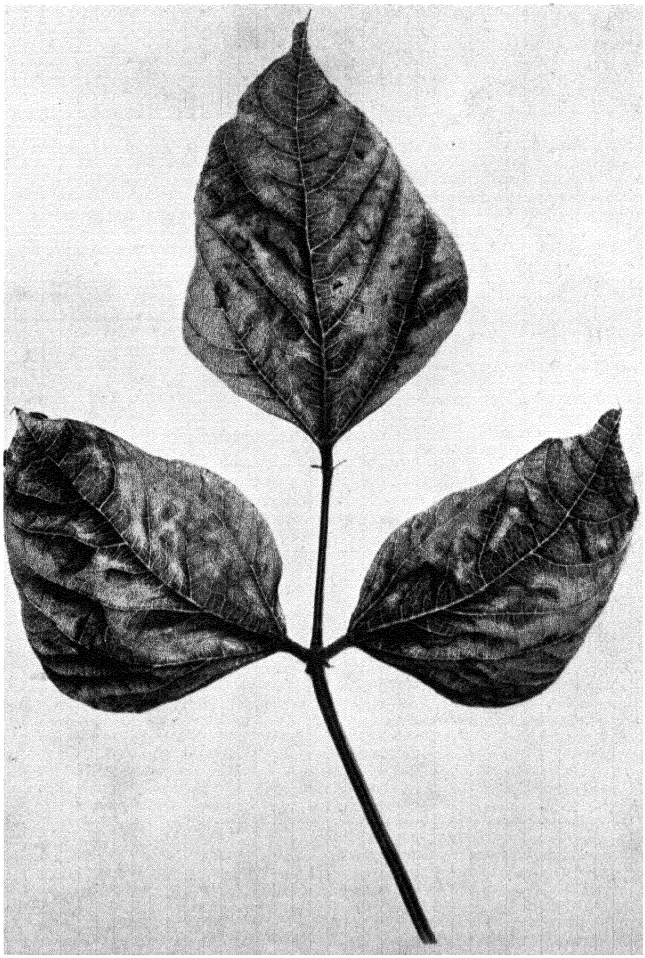
A VIRUS extract in its crude state is the liquid expressed from ground-up diseased tissue. The virus present in this liquid shows certain reactions to external influences such as heat and dilution, and these qualities are always standard for a particular virus. There is, however, some indication that they may vary slightly with different host plants.

Studies on the Effect of Heat. The effect of heat is studied by placing a little virus extract (about 5 c.c.) in a test tube and placing it in a bath of water at the required temperature. A thermostat should be used to control the heating apparatus, and the water should be well stirred. The tube should be removed after 10 minutes, rapidly cooled by running water round the outside, and the contents used immediately to inoculate healthy plants, whose subsequent state of health will indicate whether inactivation has taken place or not. Several series with a different temperature for each should be tried and it is usually suitable to allow intervals of 10° C. between the temperatures. Viruses vary greatly in their capacity to withstand this exposure to heat as the following table shows.

Thermal Inactivation Temperatures of some Virus Extracts.

Crinkle Mosaic of Potato	43° C.
Dock Mosaic	80° C.
Tobacco Mosaic	90° C. when undiluted, 82–4° C. when diluted 1 in 100.
Tobacco Ringspot	60–70° C.
Tomato Spotted Wilt	42° C.
Tomato Streak	80° C.

The Concentration of the Virus Extract. Dilution of the virus is achieved by taking one volume of the crude



BEAN MOSAIC

extract and adding it to nine volumes of water in a test tube. Sterile pipettes and tubes should be used. This process will result in a dilution of 1 in 10. Further dilutions may be prepared by a similar method, transferring one volume of the 1 in 10 strength to nine volumes of water to make a dilution of 1 in 100, and so on. The infectivity of each dilution may be tested by inoculating healthy plants in the usual way (p. 72). Tobacco Mosaic will stand dilution to 1 in 100,000 and still be infectious, whilst dilution to 1 in 1,000,000 reduces its chances to infect below those given by the ordinary method of inoculation. Many viruses are not infectious when diluted more than 1 in 100 (e.g. Dock Mosaic).

The concentration of the virus can also be estimated by a method which produces local lesions on infected leaves. Dr. F. O. Holmes found that if a piece of host-plant tissue was pounded within a piece of cheese-cloth which was then rubbed lightly on a leaf of Turkish tobacco or *Nicotiana glutinosa*, local lesions appeared on the inoculated leaf in numbers proportional to the concentration of the virus in the original leaf-part. Leaves of Turkish tobacco required treatment with iodine before the lesions were demonstrable (179).

Inactivation by Disinfectants, &c. The effect of chemicals and disinfectants on the virus extract is studied by substituting the crude liquid for water in the preparation of the strength required. The effect of 70 per cent. alcohol would be seen, for instance, if 7 c.c. of absolute alcohol were mixed with 3 c.c. of extract, or the effect of 2 per cent. formalin by adding 0.2 c.c. of 40 per cent. formalin to 9.8 c.c. of extract. These mixtures are inoculated after standing for half an hour. The resistance of many viruses to the inactivating influence of various chemicals is shown by the table:

Virus Inactivation by Chemicals.

Ordinary Tobacco Mosaic Virus	Destroyed by 80% alcohol, 40% formalin, 1% nicotine, 1% atropine, 5% digitalin, 2% oil of mustard. Not destroyed by ether, carbon tetrachloride, toluene, many inorganic and organic acids and salts.
Yellow Tobacco Mosaic	60% alcohol, or 1 in 200 nitric acid does not kill in 1 day.
Crinkle Mosaic of Potato	Inactivated by 25% alcohol, or 1 in 500 nitric acid in 1 hour.
Bean Mosaic	Inactivated by 25% alcohol in 30 minutes.
Dock Mosaic	Inactivated by 2% formalin in 30 minutes. Not inactivated by 95% alcohol in the same time.

See also (196, 377).

Attenuation by Oxygen. If an extract of Tobacco Mosaic virus is placed in a glass tube $15 \times \frac{1}{2}$ in. filled with glass beads, air or oxygen can be slowly bubbled through it from a tube passing down the container tube and delivering near the bottom. This process results in a permanent attenuation of the virus extract after the experiment has been in operation for about fourteen days. Such attenuated virus, when inoculated to healthy tobacco plants, will produce symptoms very much milder than normal and they will remain mild through several transfers to further plants (195).

Ultra-Violet and Dark Ground Photography. No definite particles of virus can be seen when a diseased extract is photographed through a high power microscope with quartz lenses, by means of ultra-violet light, or by direct vision with an ordinary microscope and high power dark ground illuminator (176). Both these methods would show up particles of a diameter greater than half the wave-

length of the light used. The virus of Tobacco Mosaic is suggested to be about 15–20 $\mu\mu$[1] across (225), and since ultra-violet light has a wave-length in the region of 280–290 $\mu\mu$, it seems that the method is not likely to be successful. Moreover, ultra-violet light will inactivate a thin film of purified extract under a quartz plate in 15 seconds (12, 13).

Ageing of the Virus Extract. A virus extract will often remain active for a considerable period after separation from the plant. Ordinary Tobacco Mosaic will retain its activity for several years when stored in a bottle, but many viruses (e.g. Raspberry Mosaic) will not tolerate even a few minutes' separation from their hosts. It may be useful in such cases to grind up the plant tissue under thick paraffin in a mortar. This excludes air from the mass and prevents oxidation. Even this may not be sufficient to prevent inactivation.

It is a general rule that ageing in the actual tissues preserves the virus longer than storing in extract form. Diseased leaves may be dried and then crumbled to a powder, which should be kept in a well-corked bottle. A herbarium specimen of diseased tobacco, which had been in a museum for nineteen years, still produced disease when a small piece was ground in a mortar with a little water and the juice used to inoculate healthy plants.

Longevity of Virus Extracts.

Potato virus X	6 weeks.
Yellow Dwarf of Onions	112 hours.
Yellow Mosaic of Tobacco	3 months.
Cucumber Mosaic	Less than 3 days.
Potato virus Y	24 hours.
Tobacco Spot Necrosis	14 days.
Tomato Aucuba Mosaic	More than 1 year.
Tomato Spotted Wilt	4–6 hours.

[1] 1 $\mu\mu$ = a millionth of a millimetre.

Inactivation by High Pressure and Pulverisation of the Host Tissue. The virus of Tobacco Mosaic is inactivated when it is subjected to a pressure of 130,000 lb. per square inch. This is higher than the pressure required to inactivate most bacteria, but lower than that required to kill *Bacillus subtilis* (141). The virus of Tobacco Mosaic is also inactivated by thorough pulverisation of the host tissue (287).

Filtration. Beijerinck (23) was the first to direct the attention of botanists to the fact that the causal agent of Tobacco Mosaic was capable of passing through a filter candle which would hold back the smallest known bacteria. This is the true test for the inclusion of an infectious malady as a virus disease. It should be pointed out, however, that many diseases cannot be so tested owing to practical difficulties which will be discussed later in this chapter.

The filters most suitable for work on plant viroses are Berkfeld and Pasteur-Chamberland candles, and graded collodion membranes. The standard Berkfeld filters obtainable in England are rather coarse for this work, but the grades W, N, and V made in America provide useful gradations. The porosities L1 and L3 of the Pasteur-Chamberland series are useful for preliminary filters and the grade L5 gives absolute bacterial sterility. The pore size of collodion membranes can be graduated (111), and no complications are introduced through the thickness of the filter. The references (207) and (322) give further information on filters in general.

Virus extract is obtained in the usual way, and the pulp is squeezed in cheese-cloth to separate the liquid. A fine meat mincer is very useful if a large quantity of sap is to be prepared. The liquid contains large quantities of protoplasm and chloroplasts, and could not be filtered

directly without clogging the filter. It must first be passed through a clarifying filter. This consists of a wide glass tube about an inch in diameter, fitted at the bottom with a rubber stopper and glass tube which delivers into a filter flask. A bit of fine gauze is laid above the stopper, and then a layer of washed silver sand about one inch deep is introduced. A pulp prepared by mulching filter paper in warm water is then poured in above the sand, so that it will form a layer 3 inches deep when all the water has drained through. Another layer of sand should be followed by another layer of pulp. It is advisable to put on the latter in two separate layers so that the top one may be removed if it gets clogged during the process of filtration. The water must be poured out of the filter flask which may now be connected through a trap to a water pump. The crude extract is placed in the top of the tube and sucked through into the filter flask, the first drops being rejected. A light amber colour should characterize the clear sap after clarification. It is now ready to go through the candle, either through the finest grade directly, or through a coarse one first. The procedure is the same in each case.

The candle may be pushed through a hole in a rubber bung so that the liquid to be filtered can be placed inside it. The lower part of the candle should enter an apparatus for catching the filtrate under sterile conditions (Fig. 4 A). A stop-cock at the bottom of the apparatus should communicate with a veiled delivery tube. The whole filtering apparatus should be wrapped in paper and sterilized, the open ends all being plugged with cotton wool. If a Berkfeld candle is used, it should be surrounded by a glass mantle for containing the liquid to be filtered (Fig. 4 C), and provided with a delivery tube which should enter the apparatus described above, in place of the Pasteur-Chamberland candle.

The process of filtration may be hastened by connecting

the receiving tube to a water pump through a trap, so arranged that the suction of the water pump can be disconnected by opening a tap in the top of the trap (Fig. 4 B).

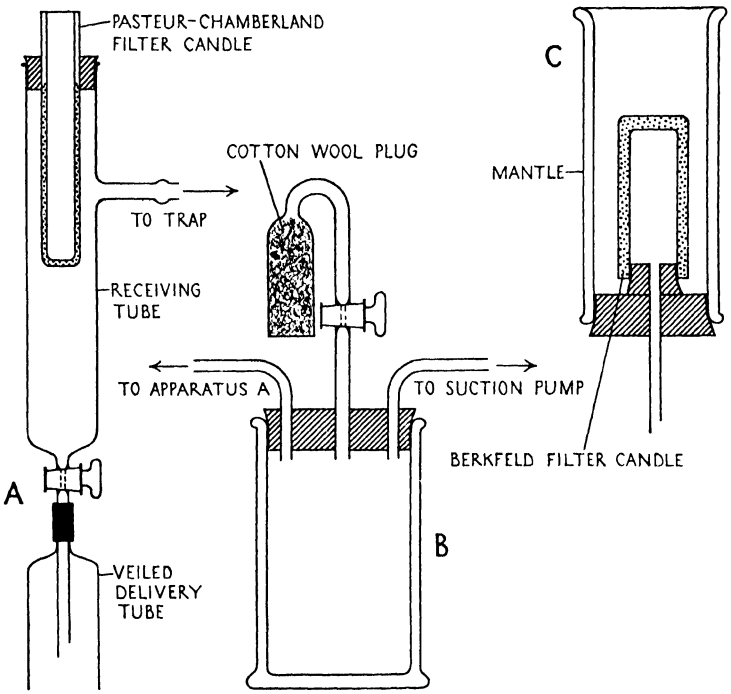


FIG. 4. A. An apparatus for collecting filtrate under sterile conditions. B. A trap suitable for use with a water suction pump. C. A Berkfeld filter candle surrounded with a glass mantle and provided with a delivery tube.

The filter apparatus, when set up, should be connected with the trap after the flaming and removal of the cotton wool plugs. The clarified sap should be poured into the candle, or mantle, and suction applied. When sufficient liquid has filtered through into the receiving tube, the tap on the trap should be opened to admit air, when the filtrate may be run off into sterile, plugged test tubes.

There will be no contaminated tubes if the plugs are carefully flamed before removal as in bacterial practice, if no filtrate is allowed to touch the sides of the veiled tube, and if the bench and its apparatus are wiped with a damp cloth just as is done before commencing bacterial experiments.

The filter apparatus must be cleaned immediately after use, the candle being boiled half an hour in water, and then heated to redness in a muffle furnace or sterilized with strong caustic soda (p. 70).

The filtrate can now be used to inoculate healthy plants, and it should be used directly, for some viruses are destroyed by standing in air for a short time. The whole process of filtration should be performed as quickly as possible after the removal of diseased leaves from the plant.

It is almost impossible to carry out filtration without diluting the virus to some extent. Virus is adsorbed by the residue on the filter and by the filter itself. The filtrate may be only one-thousandth as concentrated as the original extract. The degree of dilution varies but is not often less than one hundredth. We thus see that the direct test of filterability cannot be applied to all viruses, for some of them are unable to withstand dilution more than 1 in 100.

The method of filtration has been used by Dr. Duggar, Dr. Henderson Smith, and others to ascribe a rough size to a virus particle. After comparing the filterability of the virus of Tobacco Mosaic with that of a 1 per cent. haemoglobin solution, the first-mentioned worker suggested that virus particles were about the size of the haemoglobin aggregates—or something in the region of 40 $\mu\mu$,[1] or about a twentieth the size of the average bacterium (105). Dr. Henderson Smith and his colleague used collodion membranes and suggested that the virus of Tobacco Mosaic was about 15–20 $\mu\mu$ in size (225).

[1] 1 $\mu\mu$ = a millionth of a millimetre.

The Filtration of some Virus Extracts.

Tobacco Ringspot passes a Berkfeld W filter if previously clarified. Does not pass a Berkfeld N candle.

Tomato Mosaic passes all grades of Pasteur-Chamberland candles from L1 to L13.

Bean Mosaic will not retain infectivity after passage through any grade of Berkfeld filter.

Potato virus X passes a Pasteur-Chamberland L3 candle.

Potato virus Y does not pass an L3 candle.

The Particulate Nature of the Virus. The demonstration that a virus consists of a series of particles came first from Iwanowsky, who showed that water and salt solutions could penetrate a freshly prepared 2 per cent. agar gel, but the virus extract could not. It could do so, however, after ten days when the structure of the gel became more open. Indian ink, which is a suspension of very fine particles, could not penetrate fresh agar, but did so after ten days, so, by analogy, the virus was deemed to be of a particulate nature (184).

Purification of the Virus Extract. Most of the work bearing on the properties of the virus has been done on the crude extract. This contains many substances other than virus, and it is possible that these may affect such qualities as resistance to heat or ageing. Workers on the problem have therefore tried to obtain the virus in a pure state. The general methods are set forth below.

Filtration has already been discussed. It removes the protoplasm and chlorophyll but leaves an amber-coloured extract which is not very pure. The method has also the disadvantage of diluting the extract.

Collodion membranes of graded pore size can be used to concentrate a virus extract (111). If the virus is filtered through a membrane which allows virus particles to pass, and then through another which holds them back, the

supernatant liquid of the second filter will contain a relatively high concentration of virus.

Centrifugalization. This is a more successful method of purification, in that the debris is removed from suspension, and does not collect in a coat which exercises adsorptive action on the virus (246, 247). A force equivalent to 70,000 times gravity is applied by a super-centrifuge running at a speed of 35,000 to 40,000 revs. per min., with a bowl 3 inches in diameter. Whilst the method results in less dilution it does not give a very pure extract.

Adsorption and Reprecipitation. The virus can be adsorbed from an extract by such agents as charcoal or calcium phosphate. The adsorbing agent is removed and washed, after which the virus is liberated by passing carbon dioxide into water containing the treated material. This results in a water-clear liquid which is infectious (49, 50).

The precipitate obtained when safranin is added to juice from a diseased plant contains the virus. It may be washed, and the safranin decomposed by Lloyd's alkaloidal reagent, when the supernatant liquid contains virus in greater concentration than the original juice (419).

Guttation. The drops of water exuded from the hydathodes of certain virus-infected plants, when induced by the application of pressures over 30 lb. per square inch to the base of the stem, are capable of producing infection when inoculated to healthy plants. The liquid, however, often contains traces of protein and inorganic salts. Naturally induced water of guttation is not infectious (153).

The Removal of Plant Proteins. This is effected by centrifugalization and filtration, but it can also be done by keeping the extract one or two degrees above freezing point for several days.

The Chemical Nature of the Virus. Owing to the difficulty of obtaining pure virus, its chemical nature is

not easy to determine. A virus extract of Tobacco Mosaic is inactivated by treatment with 70 per cent. alcohol or acetone, but the residue produced remains infectious. The infectious agent is adsorbed from suspension by a solution of safranin (422), and from its heat coagulation reactions it seems very likely that the virus is protein-like. If virus extract is treated with lead acetate solution, the precipitate contains the virus, which may be liberated by treatment with potassium hydrogen phosphate after washing with potassium orthophosphate. The resulting solution contains Kjeldahl nitrogen (421).

Attempted Cultivation of the Virus *in vitro*. Plant viruses cannot be cultivated away from their living hosts. Attempts have been made to cause them to multiply in plant extracts prepared in a variety of ways, but all the results, with the exception of one claim (284), have been either negative or inconclusive (150, 153, 264, 284, 286). Many animal viruses are amenable to tissue-culture. A piece of the host tissue is removed and kept alive in physiological salt solution, when the virus will multiply within it. Plant viruses behave similarly in that the virus of Tobacco Mosaic has been shown to increase in detached tobacco leaves (154, 306).

The Electrical Charge of Virus Particles. Virus particles are negative in charge. They migrate to the anode in solutions of pH between 4 and 9 (400). See also (288).

IV

THE RELATION OF INSECTS TO VIRUS DISEASES

General Characters of Insect Transmission

It is possible that insects are responsible for most of the spread of virus maladies which takes place when conditions are not controlled by human agency. Their activity in spreading these diseases is now well established, and it is known that Aphids, Jassids, Capsids, Leaf-hoppers, Grasshoppers, and other families are capable of playing this part. A particular species of insect may be capable of transmitting more than one virus disease. The aphis *Myzus persicae* Sulz. has been shown to be capable of spreading no less than fourteen distinct viruses. Some diseases, however, as Aster Yellows and Banana Bunchy Top appear to be transmitted by only one species of insect. The leaf-hopper *Cicadula sexnotata* spreads the former, whilst the aphid *Pentalonia nigronervosa* seems to have specific relationship with the latter. Many other diseases are apparently disseminated by one specific insect, but it should be borne in mind that further knowledge may dispel this idea. There is certainly a degree of specificity, for the aphid *Myzus persicae*, which transmits many viruses, will only take Cucumber Mosaic from a tobacco plant infected with a mixture of this malady and Ordinary Tobacco Mosaic. The latter disease is apparently never transmitted by this aphid (171). In the case of Aster Yellows, the transmitting Cicadula is not the species of insect most abundant on asters, and this fact lends further support to the idea of specificity.

A curious case of differential transmission has been recorded by Dr. K. M. Smith, who found that infection

by means of a needle-prick produced different symptoms from those induced by insect transference of the same disease. The insect in question was *Myzus persicae*, and the disease Potato Crinkle, now known to be caused by a mixture of viruses, different combinations being carried by the needle and by the insect (p. 7).

Aphids may undergo a process of ecdysis or skin-shedding, whilst feeding on plants. An insect which has been feeding on a virus-diseased plant and has become viriferous does not lose this capacity by the loss of its outer covering. When reproduction of viriferous aphids takes place by the budding-off of nymphs from the wingless females (the stem mothers or fundatrices), the offspring do not inherit this capacity from their mothers, nor are the eggs laid by virus-bearing winged females, or any stages hatched from them, capable of producing infection unless re-fed on diseased plants (115 *et al.*). It is, in general, only the mature insect which transmits disease, though instances have been described (17 and 220) where the larval stage alone was capable of becoming viriferous. The adult retained the ability to transmit virus, but could not itself attain it. The larval forms of some insects such as the *Tipulidae*, which spend this stage in the ground, have been shown (115) to transmit virus from plant to plant.

Cicadula sexnotata evinces a very close relationship with the virus it transmits. An incubation period of the virus on the insect must elapse before the leaf hopper becomes viriferous (214). This means that a period of usually seven to fourteen days must pass after the insect first feeds on a diseased plant before it is able to transmit that disease to a healthy plant. The reason for this is not yet known, but the phenomenon has been observed for the transmission of some potato viroses by *Myzus persicae*, and also for other virus-insect relationships.

Insects which pierce the tissues of their host-plants do so

by means of the insertion of a proboscis, through which they secrete salivary juice. This mixes with the plant sap, and the mixture is subsequently drawn back into the animal's body. It goes into the alimentary system, and it is probable that the time taken for the virus to be absorbed into the insect's body and permeate as far as the salivary glands represents the incubation period of the virus in the insect. It must be because the salivary juice is not all re-absorbed from the plant that the latter becomes infected if the aphids feeding on it are viriferous. Insect transmission is the most efficient means of infecting with virus disease, and will work when other methods fail. A single aphid is usually sufficient to transmit disease, and it frequently retains its capacity to do so throughout its life, though there is great variation of this faculty. Some diseases are retained by the insect for not more than fourteen days.

It is not always possible to demonstrate a close relation between insect and virus. Transmission by biting insects probably approaches mechanical transfer. It has recently been shown (318) that Bean Mosaic may be transmitted by the pollen. This means that insects which disseminate pollen may also spread virus indirectly, and play no more part than a puff of wind or the brush used for artificial pollination.

Selected References to Insect Transmission: 87, 115, 116, 152, 171, 173, 214, 267, 270, 334, 351, 352, 353, 369, 371, 373, 376 (Rev.), 386, 392, 393, 398.

The Technique of Insect Transmission

Stocks of disease-free insects should be maintained during the summer months by feeding on healthy plants under cages. They should be obtained, if possible, early in the season from healthy plants which are not susceptible to infection by the virus disease under experiment. The

aphid *Myzus persicae* may be reared on cabbage for experiments on the transmission of Cucumber Mosaic. Many aphids, as the one just mentioned, and its sister species *M. pseudosolani*, are readily overwintered under controlled conditions. Reproduction by these species in the summer is mainly by the formation of nymphs from stem mothers (i.e. by vivipary), but towards autumn, winged males appear and viviparous females are also produced with wings. In overwintering stocks of these varieties of aphids, winged males and winged viviparous females are transferred to a winter host plant, within a cage, in a suitably heated greenhouse. Spinach will serve as a winter host of *M. persicae* whilst that of *M. pseudosolani* is *Rhamnus catharticus*. On these hosts the winged viviparous females produce oviparous females. Fertilization by the male results in the production of eggs which hatch after a suitable period. It may not always be necessary to provide an alternative host plant for the winter.

Insect Cages. The simplest type of insect cage is that shown in Fig. 5, and is, perhaps, the most generally useful. The framework is made from a single length of wire which is bent into two circles, one at each end of a straight part. The circles are bent so that they will form the ends of a cylinder. Thin, unsized calico is the best material to form the cylinder, which is then passed over the framework. It should be about twice as long as the framework. If the wire is fairly thick it will make a relatively rigid structure. The apparatus is next lowered vertically over a plant, and the lower ends of the muslin are gathered round a wad of cotton wool placed tightly round the stem. A piece of raffia or string tied round the gathered calico will make all secure. The upper loose ends of the calico should be similarly tied, and supported from a strong cane or stake. The top part of the plant will project into the cage, and if the procedure is to be carried out in the open in summer,

the whole of the aerial parts should be so enclosed. A few leaves may be left uncaged if the plant is growing in an insect-free greenhouse. Experiments may also be performed by placing a glass cylinder round a plant growing in a pot of suitable size. The bottom of the cylinder should be pressed well into the soil, whilst the top may be closed by stretching a piece of muslin over it, and securing by means of a rubber band.

Transference of Insects. Insects are transferred from one plant to another by means of a camel-hair brush. It is, perhaps, most convenient to pick a leaf from an aphid-infected host and place it in a wide glass tube, closed by a wad of cotton wool. This can easily be taken to the plant which it is desired to infect, and the insects gently brushed off with the camel-hair brush. They will cling to the brush, and may be gently wiped on to their new quarters. The brush is not usually sterilized, as it is found that this gentle treatment does not transmit the virus, not even the virulent Tobacco Mosaic. Active insects like capsids cannot, however, be handled readily by means of a brush. An apparatus consisting of two tubes about $\frac{1}{4}$ inch wide, joined by a length of rubber tubing, should then be made. One tube should be short to form a mouthpiece, whilst the other should be about 6 inches long, and drawn out to a nozzle just large enough to let the insect enter, at one

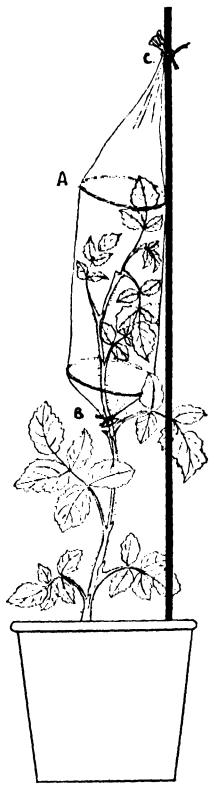


FIG. 5. Diagram showing how a healthy plant may be infected by caging aphids upon it. A. Wire framework to support the muslin cage. B and C. Open ends of the cylinder of muslin tied round wads of cotton wool.

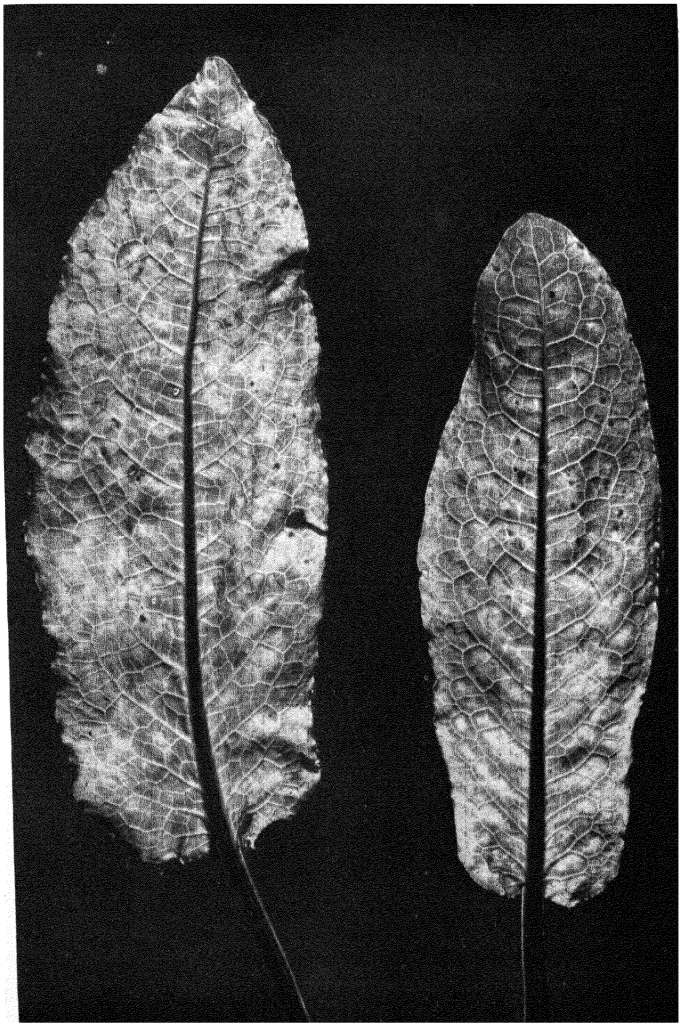
end. The nozzle end of the apparatus is to be placed near the insect, which can be sucked into the tube by a rapid inhalation, and blown on to his new host.

Aphids from healthy stock should be transferred to plants infected with the disease it is desired to transmit, and left there for about a week, being enclosed in a cage all the time. This period of feeding should be sufficient to render them viriferous. Several of the insects are then removed, and caged upon a healthy plant, whose subsequent state of health, after the lapse of a suitable incubation period, indicates the success or failure of the attempted infection.

An infected plant may become so badly infested with aphids that it can no longer make good growth. It should be temporarily removed, together with its pot and insect cage, to a small chamber where it can be fumigated with nicotine vapour. Symptoms will then develop in the normal way. At the close of an experiment the plants and aphids are burned.

It is sometimes desirable to restrict the area of feeding of a single aphid. The unfortunate animal can be enclosed in a short, wide glass cylinder, with one end closed with a wad of cotton wool. The other end is pressed against the blade of a leaf, and kept in position by a suitable spring and pad. Such technique has been used frequently in experiments on the rate of spread of virus.

Determination of the Incubation Period of the Virus on the Insect. Insects from the healthy stock should be placed in a covered glass dish until they are hungry, and then regaled upon a diseased plant within a cage. A few should be transferred therefrom every subsequent day to a cage upon a healthy plant, a fresh plant being used each day. The incubation period of the virus on the insect is not usually longer than a fortnight, so transfers may cease



DOCK MOSAIC

after that time. The state of health of the inoculated plants will show after what time the aphids became viriferous.

Insects may also be fed for a short period on a diseased host, and then removed to a healthy plant. Subsequent daily transfers will show when the insects become virus-bearing. It is not necessary for the insect to feed on the diseased plant during the whole of the incubation period.

Determination of the Duration of Virus-bearing by the Insect. Many insects, having once fed upon a diseased plant, will remain capable of transmitting disease throughout their whole lives, but others retain this capacity only for a short time. This period can be found by removing viriferous insects from a diseased plant to a cage on a healthy host. At certain recurring intervals (a fortnight is generally most suitable) single insects are removed to fresh healthy plants under cages. This process is repeated until there are no more insects to transfer, or until they are incapable of inducing infection. The experimenter should note that only mature insects are transferred each time and not nymphs which have been budded. This experiment is very similar to the last, except that the intervals between each transfer are longer.

The Preparation of Microscope Slides of Insects Feeding on Plant Tissue. A solution of 70 per cent. alcohol in water is saturated with mercuric chloride, and then about four drops of strong acetic acid are added to every 100 c.c. The mixture is boiled immediately before use, and then a leaf or stem with insects feeding thereon is dropped into the boiling liquid. Care must be taken not to let the alcohol vapour catch fire. After cooling, the preparation is taken through alcohols of increasing strength, embedded in paraffin and sectioned by the microtome by the usual methods.

An Insect Test for the Filter-passing Ability of Some Viruses. Some virus diseases can only be transmitted readily through the agency of insects. It is difficult to show that such viruses are capable of passing through fine filter candles, but an attempt may be made in the following manner (65): Filter the extract in the usual way (p. 28), and add a strong, syrupy solution of cane sugar to the filtrate. Pour this mixture into a parchment sac, and suspend it in a glass jar enclosing the transmitting insects. These will feed from the liquid in the bag and may in due course become viriferous. It should be pointed out that this is not strictly a 'biological' means of transfer (p. 71), as the virus is not always in contact with a living host.

Some Virus Diseases and their Insect Vectors.

Aster Yellows	*Cicadula sexnotata*, a leaf hopper.
Banana Bunchy Top	*Pentalonia nigronervosa*, an aphid.
Cucumber Mosaic	*Aphis gossypii*, the melon aphis. *Myzus persicae*, the peach aphis. *M. pseudosolani.* *M. circumflexus.* *Macrosiphum solanifolii*, the pink and green potato aphid. *Diabrotica vittata*, the vine beetle. *D. duodecempunctata.*
Maize Streak	*Balclutha mbila*, a leaf hopper.
Potato Leaf-roll	*Myzus persicae.* *M. pseudosolani.* *M. circumflexus.* *Macrosiphum solanifolii.* *Calocoris bipunctatus*, a capsid. *Typhlocyba ulmi.* *Aphis rhamnae.* *A. rumicis.*

	Eupteryx auratus.
	Lygus pratensis, a capsid.
	Psylliodes affinis, a flea beetle.
	Tipula paludosa (larva), a crane fly.
Potato Mosaic (complex) .	*Myzus persicae.*
	M. pseudosolani.
	Macrosiphum solanifolii.
	Aphis rhamni.
	A. rumicis.
	Mamestra brassicae.
	Psylliodes affinis.
Potato Spindle Tuber .	*Melanoplus sp.*, grasshopper.
	Epitrix cucumeris, a flea beetle.
	Systena elongata, a flea beetle.
	Disconycha triangularis, a leaf beetle.
	Lygus pratensis.
	Leptinotarsa decemlineata, the Colorado Beetle.
	Myzus persicae.
Sugar Beet Curly Top .	*Eutettix tenellus* (in U.S.A.).
	Agallia sticticollis (in the Argentine).

See also the Review (376).

V

ECONOMIC EFFECTS AND MEASURES OF CONTROL

THE economic significance of virus diseases varies greatly. It is rare to find that a disease is beneficial, but such is the case in the 'breaking' of tulips, a virus disease which causes the flowers to become striped, dashed, or flecked, instead of being self-coloured. The virus-infected flowers are very beautiful, and are greatly appreciated by many people. The variegations of some of our garden shrubs, as *Abutilon*, and *Euonymus*, are due to an agent which is infectious, and most probably a virus.

The balance of the effect of virus is very greatly on the debit side, however, for those maladies which affect market crops usually cause severe damage. Tobacco may be depreciated about 55 per cent. in yield and quality together, and care in the transplanting and 'bugging' seasons is usually well worth while. The greatest damage caused by virus diseases is perhaps the effect of the so-called 'degeneration' on the potato crop. Some idea of the reduction in yield caused by particular viruses can be obtained from Table I, which shows the average yields of about 40 plants of each kind, grown on heavy land not particularly suited for potato growing. Table II shows the relative yields of healthy and diseased plants grown on a light soil more suitable for potato culture.

TABLE I

Average Weight of Produce per Plant on Heavy Soil.

Variety	*Healthy*	*Interveinal Mosaic*	*Crinkle*	*Leaf-roll*
	lb. *oz.*	*lb.* *oz.*	*lb.* *oz.*	*lb.* *oz.*
President . . .	1 13	1 8½	1 8½	
Majestic . . .	1 14	1 9	10½	
Arran Victory . .	1 14	1 3½		15

TABLE II

Average Weight of Produce per Plant on Light Soil.

Variety	*Healthy*		*Mild Mosaic*		*Leaf-roll*	
	lb.	*oz.*	*lb.*	*oz.*	*lb.*	*oz.*
King Edward . . .	3	8				13
Majestic	4	3	2	15		

Seeing that virus diseases cause such drastic reduction in the yields of individual plants, it follows that the more diseased plants there are in a crop the lower will be the yield. Insects spread the diseases from plant to plant in the field. The potato-growing districts of England have large numbers of insects, particularly aphids, and virus diseases are there spread quickly. The stocks rapidly become totally infected with the diseases, the yield falls, and the growers must obtain fresh stocks from areas where insects are relatively scarce, viz. Eastern Scotland or Northern Ireland. Nevertheless, ordinary Scotch seed frequently contains up to about 30 per cent. of virus-bearing tubers, and rarely has less than 12 per cent. English growers, therefore, start with a considerable amount of disease in their stocks, and it is greatly to the credit of the more progressive Scotch and Irish seed raisers that they have put on the market virus-free potato seed before most English growers have realized the need for it. Such virus-free seed has been given the rather inappropriate name of 'Stock Seed', and is certified by the respective Government Departments of Agriculture as having less than 1 per cent. of virus disease.

The following typical experiment shows the beneficial results of using virus-free potato setts. Duplicate plots on the same field were planted with (*a*) Stock seed, (*b*) Ordinary Scotch seed, and (*c*) Seed thrice grown in the district (Thirsk, Yorks.). The variety was Arran Consul and the different plots were planted at the same time.

The Stock seed produced a crop which had, on an average, one virus-diseased plant in every long row (about 270 ft.). The ordinary Scotch seed had twenty-two diseased plants in the same length, whilst the thrice grown stock had ninety-five virus infected plants per row. These differences are reflected in the average yields (expressed in tons per acre):

TABLE III

Stock seed	*Ordinary Scotch seed*	*Thrice grown*
12 tons per acre.	11 tons 4 cwts. p.a.	6 tons 10 cwts. p.a.

There is thus an increased yield of 16 cwt. per acre in the first season due to the relative freedom from virus disease. The few diseased plants were removed from the Stock seed plots so that seed from them should be still relatively free from virus next year. Virus-free stocks of potatoes can be planted year after year in Yorkshire if this simple procedure is followed. The diseases most prevalent in this experiment were Leaf-roll, Interveinal Mosaic, and Crinkle.

New Stock seed costs a pound or two more per ton than ordinary Scotch seed, but with reasonable care in rogueing it can be grown for at least twice as long as ordinary seed in a lowland area. A saving on the cost of seed alone will therefore be obtained, and the increased yield will be felt each year. Stock seed can be obtained from many growers. Lists of certified stocks are compiled by the Board of Agriculture for Scotland, the Department of Lands and Agriculture for the Irish Free State, and the Ministry of Agriculture for Northern Ireland.

The losses sustained by potato growers can be paralleled on many other crops. Commercial stocks of raspberries in England are probably nearly all infected with a leaf-chlorosis, whilst hops are subject to a virus disease. Strawberries, black currants, and runner beans are likewise

infected, each with their own particular malady. All these virus diseases are real menaces to the cultivation of their respective hosts. It is one of the ironies of Nature that a Mosaic Disease of the dock scarcely handicaps this weed, though several virus diseases are known which ultimately kill their cultivated hosts. America has a wide range of viruses—on sugar cane, lettuce, beans, tomatoes, sugar beet, wheat and other cereals, crucifers, potatoes, and many other hosts. The potato crop is reduced in yield only by about 3 per cent., for most varieties are carriers, and the high summer temperatures usually mask the symptoms and reduce the damage.

Methods of Control. No cure has so far been found for any plant infected with a virus disease (e.g. 32, 320), but there are several lines of attack to decrease their harmful effects. These are set forth below so that the practical grower may select those most suited to his particular conditions.

1. *Rogueing.* This is the *complete* removal of infected plants from a population as soon as symptoms proclaim them to be diseased. Experiments by numerous workers have shown that it is not effective unless the neighbours of the offending plant are also removed. This is because a plant becomes infected several days before showing symptoms, and during this period insects may transmit the disease to the surrounding plants which may not show symptoms when the first-infected plant is removed. Rogueing should never be done when the temperature of the atmosphere is higher than the symptom-masking temperature of the virus in question. It is a method which should only be used when there are relatively few diseased plants.

2. *The use of resistant varieties.* Virus diseases which produce mottling of the leaves usually work mischief on

their host by causing decreased carbon assimilation, and, therefore, decreased growth and food storage. The severity of mottling is not always uniform on all varieties of a species, and it may be profitable to grow those which exhibit least disturbance in this respect. The method is poor in practice, for the disease spreads rapidly until all the plants are infected. They then provide a source of the disease for any more susceptible plants in the neighbourhood. The potato King Edward is an example of a resistant variety since it shows only a peculiar curling of the young leaves when affected with the leaf-mottling disease Crinkle.

3. *The use of Carrier varieties.* These are varieties which do not show any symptoms whatever, but nevertheless contain or 'carry' the virus in a virulent form. The inoculation of juice from them to a susceptible variety would produce disease in the latter. They usually show no reduction in yield, but are a great menace to susceptible plants in the neighbourhood, as the virus may be readily transferred. The potato variety British Queen is a carrier of a disease which appears when inoculation is made to healthy tobacco. The symptoms on tobacco are at first very slight, but become more severe with successive transfers on this host.

4. *The use of Immune varieties.* This presents the best practical solution to the problem of the control of virus diseases, but such varieties are unfortunately rare. Many supposed immune plants are later found to be carriers, and in any case the evidence for immunity is largely based on negative transmissions. A true immune plant should not show any disease when its juice is inoculated on to a healthy susceptible plant. Varieties of the bean (*Phaseolus vulgaris*) and of sugar cane have been reported as being immune to their respective mosaic diseases.

5. *Vegetatively propagated stocks should be free from disease.* Vegetative propagation is the process of severing part of

a plant and putting it under conditions where it will regenerate a new and complete individual. This will be a part of the parent plant. Since most viruses spread to all parts of their hosts (p. 18), it is not surprising that vegetative propagation is one of the most extensive methods of spread with which a grower has to contend. It is equally obvious that the vegetative propagation of healthy plants will produce a disease-free clone, unless infected by some other agency. Plants which show no symptoms and are at a distance of twenty yards or more from any diseased plants (to guard against insect spread) must be taken, and the perennating portions stored in a place free from insects. They should be tested if possible by indexing (see below), or by inoculation to healthy varieties in order to guard against the propagation of carriers. Further growth should take place in an insect-free environment (preferably a greenhouse with 32-mesh gauze over the ventilators), until multiplication has progressed far enough to plant out the stock in the field. A place which is relatively free from insect vectors should be chosen. Upland areas may provide this freedom, and yet allow good growth, or small 'islands' may be planted in a crop of ensilage or cereals. Care should be taken that the nearest stocks are at least twenty yards from the selected ones, which should, moreover, be harvested before insects become abundant in the area.

The actual details of the process of selection and isolation must be worked out by each individual investigator. He must have regard to the climate of his district, and its indirect effect on insect life. If a district is naturally free from vectors, as happens with the potato districts of the north-east coast of Scotland, it is not necessary to harvest the plants in an unripe state, and they can be grown to maturity (128, 289, 379).

6. *Indexing* is the growing of one bud separated from

a tuber or other vegetative organ in such an environment that it begins growth precociously. The shoot which it produces will show, by the presence or absence of symptoms, whether the original tuber was healthy or not (33, 206). The buds or 'indexes' are cut to include at least ½ inch depth of tuber, and the knife is sterilized by wiping with a rag steeped in weak Lysol solution between each tuber. Four-inch pots are large enough to receive the index-pieces, and they should be kept in a greenhouse at 65° F. The operation should begin in early winter so that planting stocks can be ready for spring. Should any diseased plants appear, the tubers which contained the respective buds are thrown into a furnace, leaving the remaining stocks of tubers free from disease.

There are several criticisms of the method. It is taken for granted that the small piece of tuber included with the bud is an index of the state of health of the whole tuber, but this is not always true. If, for instance, infection of a potato plant occurs late in the season of growth, the virus may not move into the whole of one tuber. Several instances where the index bud produced two shoots, one apparently healthy and the other diseased, have been noted. The virus can be recovered from the seemingly healthy shoot, but the state of health of the whole tuber could not have been judged accurately from the absence of symptoms on this shoot alone.

The method of indexing is widely used in America, where it has undoubtedly resulted in the production of extensive stocks of potatoes with little disease. Dr. Salaman, in our own island, uses a slight modification, with considerable success. He takes out the index by means of a cork-borer, and thereby obtains a sample more representative of the whole tuber.

The process of indexing demands a good knowledge of the varieties which are carriers of the disease to be

eliminated. The absence of symptoms from an index plant can only be significant if it is known to which diseases the variety is susceptible.

7. *The use of seed propagation.* The virus of some diseases does not pass to the embryos of their host plants, though (as in Tobacco Mosaic) it may be present in the ovary walls and calyces. This provides a ready means of raising disease-free plants, for reasonable care expended to free the seed from all chaff and flower stalks will achieve this result. Some virus diseases, as the Mosaics of Bean and Lettuce, are transmitted from one generation to the next through the embryo. In these cases, seed should be saved only from disease-free plants.

A plant stock which is normally propagated vegetatively is, as has been shown, likely to become infected very rapidly with virus disease. The production of plants from seed from such stocks would probably result in healthy seedlings. The latter, however, may not be true to type. Varieties of potatoes are, in the first instance, raised from true seed, but have to be kept true to type by tuber propagation. The old idea that the life of a potato variety was about twenty years meant that when the variety was initially raised from seed, it was free from disease, but the commercial stocks gradually became more and more diseased, until, after about twenty years, there were no disease-free plants left.

8. *The sterilization of soil.* Several viruses are capable of being transferred to a healthy plant by some agent in the soil—probably by insect larvae. All infection by this means can be eliminated by heating the soil by steam for half an hour, or by watering one ton of soil with a mixture of 1 gallon of formalin and 39 gallons of water. Soil sterilization is really only practicable in the preparation of seedbeds. Steam sterilization can be carried out without moving the soil, but for chemical treatment, it should be

removed to a stone or concrete floor. It should there be spread out in a layer 6 inches deep, and turned frequently during the watering with the formalin solution. Several weeks should elapse between treatment and seed sowing. Soil which has grown diseased plants should not again be used for raising seedlings.

9. *Fumigation* of greenhouses kills insects, and so checks the spread of disease within the houses.

10. *Plant Hygiene.* The hands of labourers who plant out and 'stop' tobacco are frequently contaminated with Tobacco Mosaic virus. They often chew tobacco and spit on their hands, thus rendering them infectious. The curing and pickling of tobacco does not always inactivate the mosaic virus. It has been found that workers who smoke cigarettes sometimes have contaminated fingers (412). The hands should be washed frequently whilst planting out, or stopping, and before commencing any experimental work. The handling of a diseased tobacco is sufficient to render the fingers viriferous. Diseased material should be burned as soon as possible, and compost heaps should be at a considerable distance from any experimental plots. Crops must be harvested thoroughly, and weeds kept down so that no plants remain to over-winter the disease. The presence of potato tubers from the previous year's crop will often start the spread of disease, if the same crop is grown the next year. The value of rotation of crops from this point of view is obvious. Tomato Mosaic is often over-wintered in America upon various wild weeds of the genus *Physalis*. Attention to general tidiness, proper rotation, and freedom from weeds will do much to control virus diseases (79, 97, 98, 139, 412).

11. *Sterilization of cutting and pruning knives.* Some diseases (e.g. Spindle Tuber of the Potato) are transmitted to healthy plants by cutting the tubers with a knife which

has just been used on a diseased tuber. The cutting knife should be sterilized periodically by wiping with a rag steeped in weak lysol.

12. *The separation of two diseases which together cause 'Streak'.* The virus which is carried by almost all American potatoes produces no serious reduction in yield. The Mosaic Disease of Tomatoes is not a deadly menace to that crop. Yet when the two diseases are both present on a tomato plant they produce a Streak Disease which is nearly always fatal (140). A remedy is found in the separation of the two crops by a distance sufficiently great to minimize insect transmission (20 yards in most districts). This applies to districts where tomatoes are grown in greenhouses, as well as to where this crop is grown out of doors.

13. *Growth at symptom-masking temperatures.* Some plants may be grown at temperatures which mask the symptoms of the disease. This reduces all immediate damage, but the plants are sources of infection to others in the neighbourhood.

VI

THE CLASSIFICATION AND DESCRIPTION OF VIRUS DISEASES

It is one of the major difficulties of the study of virus diseases of plants that although a virus is defined as an infectious, ultramicroscopic and filter-passing particle, a direct test of the latter character cannot be applied to many of the diseases. What, then, are the criteria for the admission of a disease as a virosis?

First, and foremost, the disease must be infectious. It must be possible to induce the appearance of typical symptoms upon a healthy plant by some means of transmission. This alone would be no indication that the malady was caused by a virus, for bacteria and toxins are infectious. Toxins, however, only produce their harmful effects for one transfer—there is no increase of toxic substance unless from some source outside the plant. Evidence must therefore be obtained of the multiplication of a suspected virus within the host. This can be done by using a method of inoculation which transfers infinitesimal amounts of virus, and then estimating the concentration in juice expressed from the main bulk of the plant, after a suitable period. If this method cannot be applied, the disease should be transmitted for several 'generations'. This contributes circumstantial evidence of multiplication within the host.

An attempt should always be made to determine the filterability of a virus extract through a bacteria-proof filter. If the result is positive, it provides a valuable indication of the nature of the causal agent. If it is negative, a very careful microscopic search should be made to demonstrate the absence of fungi or bacteria, both in the filtrate and in the plant. The presence of inclusion

bodies, and the existence of a masking temperature are useful guides, but both may be found, though rarely, for fungal diseases. Symptoms of the Mosaic or Yellows type are a rough indication of the nature of a disease, but the range of miscellaneous symptoms is so wide as to be of little practical use.

New viroses or infectious chloroses are being described every year, and if it should fall to the lot of a reader of this book to find such a malady, he should endeavour to describe it with some approach to the comprehensiveness of the following plan:

Description of a Virosis or Infectious Chlorosis.

Names. Give the name which is most generally accepted and all known synonyms.

1. *Symptoms on various hosts.* Give a description of the symptoms on all parts of the principal host, and follow with brief notes of any distinctive appearances on alternative hosts.

2. *List of alternate hosts.* Give a full list of the names of plants with their authorities, of any proved susceptible, resistant, or carrier varieties. A list of the plants which have been tested and proved not to show symptoms, or harbour the virus in any way, should also be given.

3. *Transmission.* A. by seed, through the soil, by contact, by needle pricking, or by tissue mutilation.

B. by grafting.

C. by insects.

(*a*) *Vector species.* Give a list of the species which transmit the disease, with the names of any insects which have been proved not to disseminate the disease.

(*b*) Incubation period of the virus in the insect.

(*c*) Durability of infection in the insect.

(*d*) Mode of puncture of the plant.

(*e*) Is there transmission to (i) the egg? (ii) the nymph?

(*f*) Are the body juices of the viriferous insect infectious?

D. Duration of the general incubation period in the host. State also the temperature of growth of the host plant.

4. *Effect on the Physiology and Anatomy of the Host.*

A. Pathological histology.

B. Inclusion Bodies. Give the host, and say whether X-bodies, striate material, or other inclusions are present.

C. Effect on Metabolism.

D. Tissues to which the virus spreads.

E. Rate of spread.

F. Any other effects.

5. *Effect of chemicals on the virus extract.* Name the chemical, its strength, and the effect after half an hour's action.

6. *The effect of dilution on the virus extract.* At what dilution inactivated.

7. *The effect of heat on the virus extract.* State the temperature and the effect of 10 minutes' exposure.

8. *Attentuation of the virus.*

A. Attenuating hosts.

B. High temperature.

C. Attenuation of the extract by oxygen.

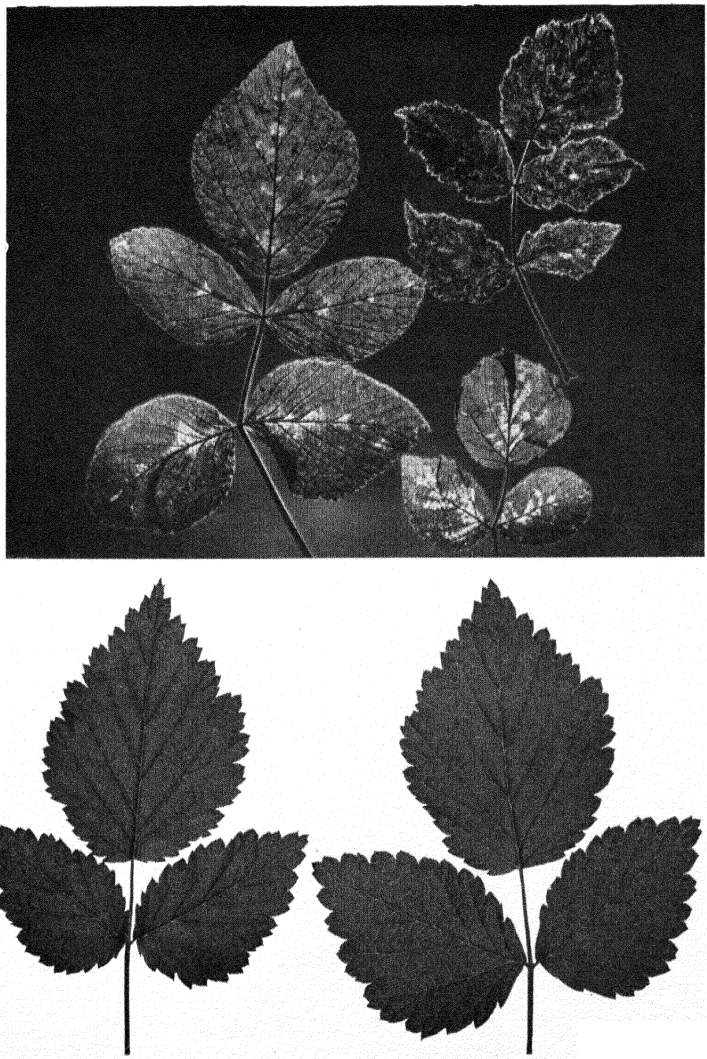
9. *Filtration.* State the grade of filter, whether the filtrate is infectious, and to what degree it is diluted.

10. *The ageing of the virus extract.* See how long the virus remains active, when stored as extract, and as dried leaves.

11. *The effect of centrifugalizing.* Give the speed and diameter of the bowl or head, the time of action, and its effect.

12. *Special characteristics and economic significance.*

It is beyond the scope of this book to describe all known viruses in detail, but a few selected ones are here dealt with in order that the student may have some acquaintance with the various characters. The review (376) gives a comprehensive list of virus diseases.



RASPBERRY MOSAIC (above) AND HEALTHY FOLIAGE
FROM WILD PLANTS (below)

THE ORDINARY MOSAIC OF TOBACCO (= *Tomato Mosaic*). (Plate I.)

Symptoms on Tobacco. See p. 5.

Tomato plants show mottling and stunting, with no stem necrosis.

Alternative Hosts. *Lycopersicon esculentum*, *Nicotiana rustica scabra*, *N. attenuata*, *N. plumbaginifolia*, *N. quadrivalis*, *N. tomentosa*, *N. Sanderae*, *N. alata grandiflora*, *N. forgetiana*, *N. paniculata*, *N. rustica humilis*, *N. rustica*, *N. glauca*, *Solanum tuberosum*, *S. nigrum*, *S. melongena*, *S. aculeatissimum*, *S. atropurpureum*, *S. carolinense*, *S. laciniatum*, *S. miniatum*, *S. rostratum*, *S. sisymbrifolium*, *Capsicum annuum*, *Physalis pubescens*, *P. alkekengi*, *P. franchetti*, *Petunia violacea*, *Nicandra physaloides*.

Negative transmissions have been reported for *Nicotiana affinis*, *N. vincaeflora*, *Phytolacca decandra*, *Phaseolus lunatus*, *P. vulgaris*, *Cucumis melo*, *C. sativus*, *Datura stramonium*, *D. meteloides*, *Solanum dulcamara*, *Rumex obtusifolius*, *Pelargonium spp.*

Transmission by soil, contact, grafting, needle pricks, tissue mutilation, by spraying or bathing the plant, but not through the seed.

Insect transmission by *Pseudococcus citri* (also by the Tobacco hornworm).

Incubation period. 3–21 days.

Microscopic anatomy. Dark green areas are normal, or have two layers of palisade cells. Nuclei tend to be smaller, and plastids larger than normal. Chlorotic areas have cuboidal palisade cells. The spongy parenchyma may be normal, or somewhat looser than usual.

X-bodies occur in epidermal, and hair cells, and all tissues except the cambium, and within 7 layers of cells

from the apex of the growing point. They are associated with the disease on *Nicotiana tabacum*, *Capsicum annuum*, *Lycopersicon esculentum*, *Physalis pubescens*, *P. franchetti*, *P. alkekengi*, *Petunia violacea*, *Hyoscymus niger*, *Nicandra physaloides*, *Solanum miniatum*, *S. atropurpureum*, *S. nigrum*, *S. cabiliense argenteum*, *S. marginatum*, *S. pyracanthum*, *S. tuberosum*. All these hosts have striate material also.

Nicotiana glauca and *Solanum laciniatum* apparently do not produce X-bodies or striate material when they are infected with the disease.

Effect of Chemicals. 50 per cent. alcohol or 50 per cent. acetone inactivated a virus extract, but the virus was in the precipitate. Formalin, 1 in 200, inactivated the virus extract. Saturated ammonium sulphate solution precipitated the virus.

Dilution. The virus extract cannot usually be diluted to more than 1 in 100,000 without losing its infectivity.

Heat inactivation. 90–98° C. for ten minutes will inactivate.

Masking temperature. 37° C.

Attenuation of the extract by bubbling oxygen for several days.

Filtration. Filtrate from a Berkefeld V, or N, or a Pasteur-Chamberland L3 is infectious, but is diluted 1 in 1,000.

Ageing. The extract remains active for several years. Dried diseased leaves remain infectious for 19 years.

Centrifugalizing at 50,000 r.p.m. for 1 min. does not inactivate the supernatant liquid.

Dock Mosaic. (Plate III.)

Symptoms on *Rumex obtusifolius*. Infected plants are not markedly stunted, and show no great variation in

flowering, seeding, rooting, or general habit of growth. The lamina of the leaf develops many areas of a greenish-yellow colour lighter than normal. Some plants have the areas confined to the spaces between the veinlets, whilst others show large patches extending over several veinlets.

Alternative hosts. *Rumex lanceolatus, R. sanguineus.*

Transmission by tissue mutilation but not by needle pricks. No seed transmission. Incubation period 16–21 days.

Insect transmission. The bean aphis (*A. rumicis*) apparently does not transmit the disease.

Effect of chemicals. 95 per cent. alcohol for half an hour did not inactivate, but 2 per cent. formalin inactivated in a similar time.

Dilution to 1 in 100 remains infectious, but 1 in 1,000 is not so.

Effect of Heat. 80° C. inactivates in 10 min., but 70° C. for the same time does not do so.

Masking by temperatures of 75° F. and over.

Filtration. The filtrates from a Jenkins, and from an English Berkefeld candle are not infectious.

Ageing. Extract is still infectious after 14 days, and dried leaves after 21 days.

Raspberry Mosaic. (Plate IV.)

Symptoms vary on different varieties. Stunting may be slight, or severe, and is directly correlated with the intensity of mottling. The chlorotic areas may be diffuse, and yellow, or may be fairly sharply defined. There may be only blistered dark green areas along the veins, the green being darker than normal. Severe attacks result in a low fruit yield of poor quality.

Alternative hosts. Blackberry? and dewberry?

Very little is known about the further properties of this virus, except that it is transmitted by some species of Aphids. No less than five different virus diseases of Raspberries are recognized in America (24).

ASTER YELLOWS.

Symptoms on Aster. The first appearance on a young plant is a slight yellowing along the veins, in the whole or part of the small leaves. This is invariable, and is a valuable diagnostic feature. Later, the whole or half of the leaf becomes chlorotic. The leaves of one sector of the plant rosette may be chlorotic, the rest being dark green. Young, severely infected leaves may be almost white, but later become somewhat green. Secondary shoots frequently arise in the axils of green leaves which were mature before the plant was infected. These branches have the characters of etiolated shoots, whilst the whole plant is stunted. The flowers tend to become green, and they and the seeds may be abnormally large, though they may also be dwarfed. Hairs on diseased flowers may develop leaf-like structures. The leaf-blades are narrower than normal; their margins may be deeply cleft, but are rarely malformed. Necrosis of the upper stem occurs in severe attacks. The root system is smaller than normal.

Alternative hosts. The disease attacks a wide variety of plants belonging to the Compositae, and also species belonging to the following Natural Orders: Dipsacaceae, Plantaginaceae, Martyniaceae, Gesneriaceae, Scrophulariaceae, Solanaceae, Labiatae, Boraginaceae, Hydrophyllaceae, Polemoniaceae, Asclepiadaceae, Primulaceae, Umbelliferae, Begoniaceae, Resedaceae, Cruciferae, Papaveraceae, Portulacaceae, Caryophyllaceae, Amarantha-ceae, Chenopodiaceae, and Polygonaceae. No member of

the Leguminosae, Rosaceae, or Gramineae has yet been infected with the disease.

Transmission by grafting and insects only. No seed transmission.

Insect vector. *Cicadula sexnotata* Fall. The following have been tried as vectors without success: *Lygus pratensis*, *Empoasca flavescens*, *Agallia sanguinolenta*, *Myzus persicae*, *Thrips tabaci*, *Trialeurodes vaporaria*, *Tetranychus telarius*, *Graphocephala coccinea*.

Incubation period of the virus in the insect. 10 days.

Durability of infection usually throughout life, but some individuals lose it in more than 100 days. The virus is not transmitted through eggs or faeces of the leaf hopper.

Necrosis may occur on the cortex of the upper stem.

LEAF-ROLL OF POTATO. (Plate VI.)

Symptoms. See p. 6.

Transmission by grafting and insects. Occasional soil transmission by *Tipula paludosa*. Occasional seed-transmission. Incubation period 14–25 days.

Insect vectors. See p. 42. *Typhlocyba solani* has also been tried, but with negative results.

Incubation period in the insect. 24–48 hours.

Durability of infection in the insect, about 10 days. The virus is not transmitted to nymphs born from viriferous females. The virus is not lost when the virus-bearing aphid casts its skin.

Necrosis of the phloem is more abundant than in healthy plants, and extends to the young sieve tubes. Starch remains in leaves kept in the dark for 2–3 days longer than in healthy leaves.

Rate of spread 12–14 in. in 10 days.

SANDAL SPIKE.

Symptoms on the Sandal tree *Santalum album*. In the early stages, one or two branches may exhibit very small leaves, separated by short internodes. These symptoms may spread gradually over the whole tree. The leaves display a tendency to stand out stiffly from the branch. Fresh leaves become smaller and smaller. The parts of a tree which are affected send out new growth in practically every month of the year, producing a structure very similar to a Witches' Broom. Infected leaves are at first pale green in colour, and later become red, owing to the accumulation of anthocyanin in the sub-epidermal cells. Flowers of infected plants exhibit a marked tendency to phyllody, the perfect flower being replaced by a tuft of leaf-like structures. In advanced stages of the disease, the haustoria and fine root ends of the plant die.

Alternative hosts. Spikes have been found on other trees.

Transmission by grafting, but not by injecting diseased juice.

Microscopic structure. Infected leaves are slightly thinner than healthy, and the mesophyll cells are less elongated. Intercellular spaces are less marked.

X-bodies have been found in diseased tissues.
Starch-accumulation occurs in affected leaves.

THE INFECTIOUS CHLOROSIS OF ABUTILON.

Symptoms. Leaves of *Abutilon Thompsonii* show sharply defined areas of chlorotic tissue, often bounded by the veins. The chlorotic parts are whitish, or yellowish-green. Flowers may become variegated, and if the disease is transferred to *Althaea rosea*, the seed-pods are variegated.

Abutilon Thompsonii is probably the diseased form of *A. striatum.*

Alternative hosts. A large number of species of *Abutilon,* and *Althaea, Lavatera, Malva, Sida,* and *Sidalcea.*

Transmission by grafting and through the seed.

Microscopical anatomy. Chlorotic areas are thinner than green parts, have cuboidal palisade cells, and loose parenchyma. Green parts may have two layers of palisade cells.

Spread of virus is controlled by ringing. The removal of successive crops of leaves may cause later leaves to remain green. Shading may inhibit the appearance of symptoms, but plants remain variegated in blue and red light.

POTATO CRINKLE—a composite virus.

Symptoms. Infected plants are usually dwarfed. The leaves show a well-marked mottle chlorosis, and the margins are lobed and wavy. Individual leaves and leaflets are also smaller than normal. It has been noticed that symptoms are rather erratic, differing with different methods of transmission. This is because the disease is caused by the combined action of two or three viruses, one of which may be retained by a particular method of transmission, and lost by another means of transfer.

Transmission. The disease may be transmitted as a whole by grafting and by certain insects, but needle inoculation often transfers the mixture incompletely. The incubation period is about 21 days.

Masking occurs at temperatures of 75° F. and over.

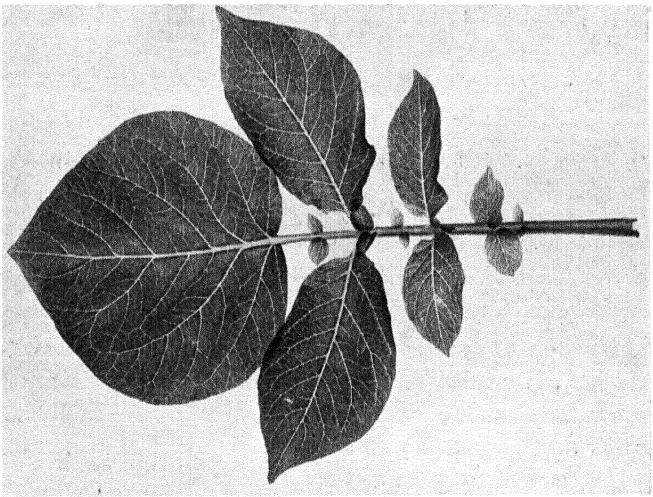
VII

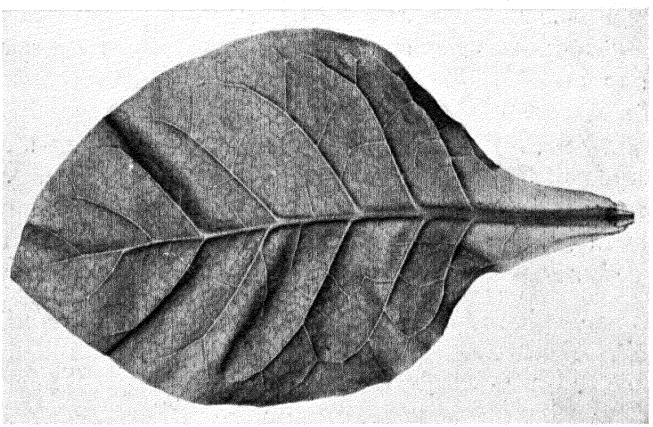
GENERAL EXPERIMENTAL WORK

THE qualifications of a good plant pathologist involve also those of the good gardener or farmer, for he must be capable of cultivating good, normal, healthy plants for his experiments. Plant viruses have not yet been cultured away from their living hosts, so the need for good, robust plants is obvious. Local practice is frequently the best guide, and it is wise for the student to incorporate the technique mentioned in this chapter with the best methods of the professional gardener.

It has been found that insects transmit many virus diseases, and it is highly probable that in Nature all these maladies are so disseminated. The growth of plants for experiment must, therefore, take place in an insect-free environment. This may be either in a selected place out of doors, or preferably in a greenhouse specially constructed to exclude insects.

The Greenhouse. The greenhouse must be provided with adequate means of ventilation; light must not be curtailed by any constructional defect. It must be painted white, and should preferably have some means of heating. It is desirable that the house should consist of several compartments, for the separate growth of healthy plants and of those infected with different diseases. Each compartment should not be less than 7 ft. 6 in. square in ground-floor area, and should have plant racks 2 ft. 9 in. high round three sides, the door being on the fourth side. The side ventilators should not be less than 2 feet square, and should be covered on the inside by a panel of copper or phosphor-bronze gauze of 32 meshes per linear inch. The panel is fixed so that when the ventilator is open it





A 'CARRIER' POTATO

The Leaf of the Potato (var. British Queen) appears quite healthy, but if juice from it is inoculated to healthy tobacco, a mild mottling appears in the latter, as shown by the lower picture. British Queen is a 'carrier' of the disease

will stop effectively the ingress of any insects. The ridge ventilators should have an area not less than the side ones, and should be similarly protected with gauze. The operating mechanism for the side ventilators should be of the knob-and-sprag type, operated totally from the outside, whilst that for the ridge ventilators should be of a type such that the operating rod may pass through a block of wood and make an insect-proof joint therewith. They should be operated separately from the inside of the greenhouse. An existing system can often be rendered insect-proof by the enclosure of the operating rod in a long, narrow bag of fine-woven cloth, of a durable nature.

The door opening should at least be provided with double doors, an inner one with panels of gauze (32 meshes per linear inch), and an outer one glazed in the usual manner. This arrangement allows the compartment to be kept cool in summer, if desired, for the outer door can be left open without interfering with the insect-proof nature of the structure. Some workers prefer to have the doors at the extremities of a small porch, so that there is less danger of insects being admitted along with the investigator on his visits to the house. One worker of the writer's acquaintance keeps an overall in the porch, to be assumed on entering, and left behind on quitting.

Greenhouse Technique. Pots should be of a size suitable to take the plant through all the stages of inoculation and incubation, as the process of 'potting-on' is apt to spread some virus diseases. A pot which has held a diseased plant may carry over small portions of infected root to infect other plants and ruin future experiments. Washing the pots and crocks is usually sufficient to eliminate contamination from this source, but sterilization with 0·5 per cent. formalin may also be practised. The pots must be left for some time after treatment with the

formalin before they are used for growing plants, and soil must never be placed in wet pots.

Soil should always be obtained from a fresh site. It should preferably not have grown the crop under experiment for at least ten years. If it is heavy or poor, it should be lightened by the addition of sand, or improved by the application of artificial manures and organic matter in the form of rotted turf or leaf mould. The two latter ingredients should come from a locality which has not grown the experimental plants for ten years previously. It is advisable to 'temper' the soil by keeping it mixed in a heap some time before use, and if it is required for the growth of plants intended for insect experiments, it should be thoroughly air-dried before potting. This process kills any insect life which may be present. The soil should be taken as far away from the greenhouse as possible when it has been removed from the pots after use. This reduces the number of possible sources of chance infection round the greenhouse. It is also beneficial to eliminate the soil as a breeding place for insects round the greenhouses by laying sand or gravel 6 inches deep for a distance of 10 feet from the walls of the building.

Chance attacks of aphids or white fly can be controlled by fumigation. Nicotine vapour will eradicate greenfly, and the vapour of tetrachlorethane or hydrocyanic acid gas will check white fly. The latter method of fumigation should only be used, except for certain crops, in an empty house. The use of a chalcid wasp parasite *Encarsia formosa* will reduce, but not control entirely, an attack of white fly.

Outdoor Plots. It may be necessary to grow certain plants out of doors in order to check results obtained in the greenhouse. When this is the case, the plants should be grown in plots separated from one another by at least

20 yards of a crop which does not harbour many insects. An oat crop or a meadow are two such surroundings. The experimenter must try to arrange his times of planting so that his plants may be well grown and ready for lifting before insects get very numerous in the neighbourhood. Potatoes must, for example, be lifted in England by the middle of July, and there are very few varieties, even late ones, which have not produced tubers sufficient for the needs of the experimenter by that date.

Hardy plants may also be grown in an elevated situation, where the transmitting agents of the virus to be studied are absent or restricted. This is more useful for the experimenter than for the commercial man, who usually finds that the altitude necessary for the degree of freedom from insects which he demands, will not allow a vigorous growth of the host plant. Such plots are usually situated on a moorland area, and may be fenced round with wire-netting whose lower edge must enter the ground 6 inches or more, to prevent the entrance of rabbits by burrowing. A shallow drain-trench round the highest and its two adjoining sides will usually provide sufficient drainage.

The coastal valleys and plains of Scotland, north of a line drawn from the Forth to the Clyde, are very suitable for the commercial propagation of virus-free potatoes. Here the increasing latitude reduces the numbers of insects, whilst potatoes grow well at any altitude up to 250 feet above sea-level.

Controls. All inoculations and experiments should be carried out against adequate controls, which should receive the same mechanical treatment as the plants to be infected, except that no virus-source is introduced to them. It is also necessary to have a considerable number of healthy plants scattered through a greenhouse or plot containing inoculated plants, so that some idea of the

incidence of chance infection, and therefore of the validity of the experimental result, may be obtained.

Fallowing or Rotation. Plots which have been used for growing a crop of plants for experiment with virus diseases should not be devoted to this purpose for two seasons in succession. They should be left fallow, or devoted to some other crop for a period long enough to ensure that subsequent experiments be not spoilt by unwanted plants left over from the previous year. This applies specially to potatoes, for even a small tuber, easily overlooked when lifting, may produce a plant next year which may possibly be a source of infection. A bad infestation of the greenhouse by white fly is best tackled in somewhat the same manner. The plants should all be cleared out and the building fumigated heavily by the cyanide method, being left quite empty for a time sufficient to starve out all stages of the insect, for want of a host plant.

Storage. Plants which are propagated vegetatively must have their dormant organs stored away from insects. This may be achieved by placing them in a cool cellar, or it may need the erection of a special storing shed. This latter should be frost proof, and may need the same precautions against the entrance of insects as were adopted for the greenhouse, viz. gauze (32 meshes per linear inch) over the ventilators and an inner door with panels of the same material. An occasional fumigation with nicotine vapour when the tubers are sprouting is a wise precautionary measure.

Sterilization. All apparatus, instruments, and the hands of the operator must be sterilized immediately after having been in contact with diseased plants or with virus extract. The methods adopted to attain this end are in general those of the bacteriologist, though no elaborate precautions

against aerial contamination are necessary. The virus is generally more resistant to germicidal chemicals than are most bacteria, so such substances must be used in strengths greater than are employed by the bacteriologist. It has been shown (p. 27) that some virus extracts can remain infectious over a period of several years. It therefore becomes imperative to sterilize apparatus and instruments immediately after they have been used, so that they will not later serve as sources of accidental infection.

There is no evidence whatever that there are sporing stages in the life histories of known plant viruses. Although many viruses require for their destruction a temperature higher than that necessary to kill most vegetative bacteria, that temperature is below the boiling-point of water. Complete bacterial sterilization is only obtained, when boiling or steaming at atmospheric pressure, by doing so on three successive occasions. The substances to be sterilized are kept at room temperatures between the steamings to induce the spores to become vegetative bacteria again. It is, however, sufficient to heat once only when utensils or instruments are to be freed from virus. Experimental work has proved that steaming for half an hour is quite effective, even when one is working with the strongest known virus—that of Ordinary Tobacco Mosaic.

Mortars and pestles, needles, pipettes, test-tubes, and similar apparatus should be freed from plant extract as soon after use as possible. Any adhering coarse plant tissue should be removed and burned in a furnace—usually that of the greenhouse heating apparatus. The utensils and instruments should then be washed free from any trace of adhering green extract. It is difficult to do this if the plant juice has once been allowed to dry, hence the necessity for its speedy removal. It is a good plan to place the cleaned apparatus in the steaming chamber and sterilize them in convenient batches, whence they may be

taken, wiped dry with a newly laundered cloth, and stored on shelves in a cupboard. The adoption of this system gives the certainty that no unsterile apparatus shall be used.

The type of steamer is immaterial. The usual type with racks or grids in a cylinder, with boiling water in the bottom, or the Arnold pattern oven are equally efficient. It is, of course, essential that the water be kept steaming vigorously throughout the whole period of sterilization. An autoclave is rarely needed by the student of virus diseases of plants.

Experiments are sometimes designed which involve the cutting of a diseased leaf. A scapel is usually employed, and can be readily sterilized by dipping the blade in methylated spirits and just igniting it in a flame. Care must be taken not to let any burning spirit drop from the blade, or the operation may become dangerous. A scalpel with a metal handle is best, but the heat must not be so intense as to cause the blade to lose its temper, and it must be allowed to cool before making further cuts.

The hands of the operator are, perhaps, the most usual sources of chance infection. They may acquire minute quantities of virus if only brushed against a diseased leaf, so they should always be sterilized after handling such material, or virus extract. Washing with soap and water will remove completely any quantity of virus, but the drying of one's hands is apt to become a problem if experimental work is at all extensive. A small linen towel may be used, and should be replaced frequently by a clean one. The crêpe paper towels which can be obtained in some localities, and which are destroyed immediately after use, solve the laundry problem. The provision of washing accommodation in or near the greenhouse is imperative.

The sterilization of filter candles is a special process. The candle should be taken from its mounting, cleaned

and boiled in water for half an hour. This removes further material from the pores. It should then be dried and heated up gradually in a muffle furnace to a dull red heat. A quarter of an hour's treatment should sterilize effectively. The candle must be allowed to cool gradually, and should be stored in a tin box or other suitable receptacle. If the candles tend to crack by heating, they may be sterilized by boiling for half an hour in strong sodium carbonate solution, and then running a large quantity of distilled water through the reverse way.

The sterilization of soil presents great difficulty. It is useless to steam it for half an hour, as the temperature of the inner masses would not rise, in all probability, above 50° C. The most satisfactory approach to virus sterility can be obtained by spreading the soil in thin layers and steaming for about two hours. The temperature of the inside masses should always be taken, and should not be less than 90° C. This temperature should be maintained for at least half an hour. It may take about an hour and a half for the inner soil to attain boiling temperature so that steaming for two hours should rid the soil of virus.

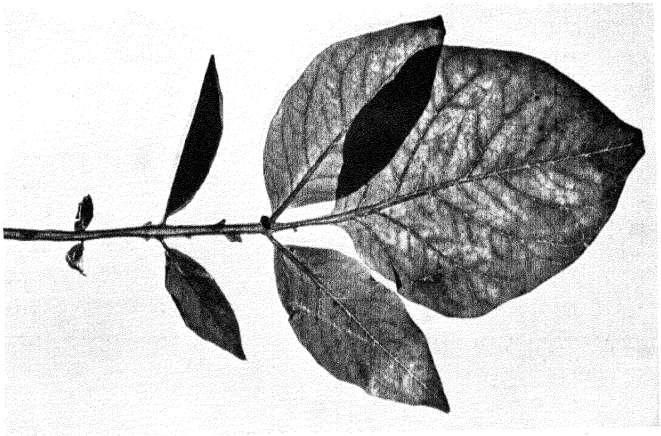
Inoculation. Inoculation is the transference of infectious material from a diseased plant to a healthy one so that the latter becomes diseased. It may be accomplished in many ways, and it has been found convenient to distinguish between 'Biological' and 'Mechanical' methods. Grafting, insect transmission, and pollination with virus-bearing pollen (318) are the chief biological ways of inoculating. The virus is never separated from a living host during the whole process of transfer. Mechanical methods are those whereby the infecting material is prepared in extract form, being taken to the healthy plant by means of some instrument or tool. This involves some wounding, however slight, of the tissues of the inoculated plant.

Methods of inoculation are not equally efficient in inducing disease in the healthy plant. There is a group of virus diseases which can only be transmitted from plant to plant by means of biological methods, whilst those viroses which are amenable to mechanical transfer vary greatly in their response to these means of inoculation. The method described later in this chapter as 'Tissue mutilation' will, in general, transmit disease better than needle inoculations. The disease of potatoes known in America as 'Crinkle Mosaic' is readily spread by tissue mutilation, but only very erratically by needle inoculation. The Ordinary Mosaic of Tobacco is transferred by all mechanical and biological methods.

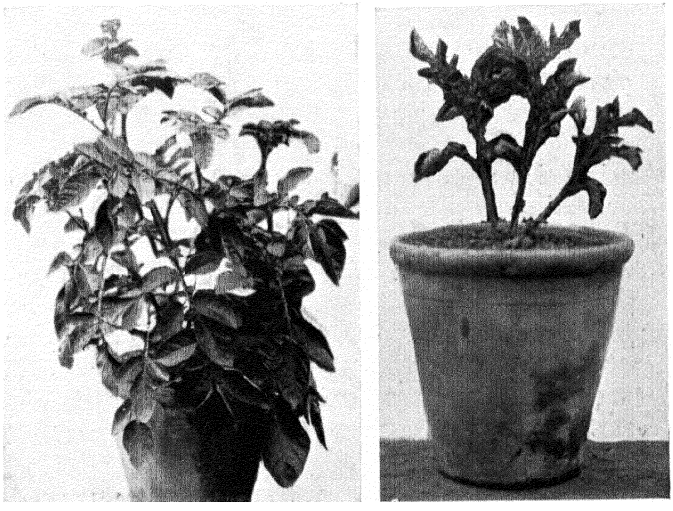
The special considerations relating to insect transmission have been treated in a separate chapter, but it is most convenient to describe the mechanical methods and grafting in this section.

Needle Inoculation. The ordinary dissecting needles mounted in wooden handles are generally used for this, the most usual method of inoculation. They are sterilized by steaming (see p. 68), and then stored in a suitable receptacle. Rust will often appear on the needle, but if not too bad, this is an advantage. The rough surface caused by the rust serves to hold a small swab of cotton wool in place. Heavy deposits must, however, be cleaned away by rubbing with fine emery paper. An extremely small amount of absorbent cotton-wool must be teased out lightly and then wrapped tightly round the needle, near the point. It is usually advantageous to prepare several more needles than appear to be required as it needs some practice to get skill in inoculation without losing the swab.

The inoculum must next be prepared. Parts of the diseased plant (usually leaves) must be separated, and ground in a glazed porcelain mortar, using a pestle of



INTERVEINAL MOSAIC. Note the interveinal mottling. Var. President



LEAF-ROLL

Left: Healthy plant. Right: Diseased plant. Note the stunted growth and rolled leaves. Var. President

similar material. A little washed silver sand may be added if the tissue cannot readily be ground. When it has been reduced to a pulp, it should be massed to one side of the mortar, and the juice drained away by pressure from the pestle. The mortar should be supported so that the juice and pulp are not in contact. The hands of the operator should then be washed, whilst the pestle should be washed and placed in the steamer ready for the next sterilization. It is important not to let the uncleaned pestle get in contact with the bench, or other working surface, as this may supply a chance source of infection.

The plants to be inoculated should be ranged in convenient fashion on the greenhouse bench. Five plants are often given the same treatment, so it is best to place them in rows of five. They should be sorted so that all the plants for one experiment are as nearly uniform in size as possible.

The mortar containing the infectious juice should be within easy reach of the operator, who should roll up his sleeves so that they do not touch the plants as he is working. A virus like that causing Tobacco Mosaic might be spread by touching a diseased or freshly inoculated leaf with the sleeve, and then drawing it over a healthy plant. A clean, unused, wooden plant label should be held in the left hand and brought against the underside of a leaf on a plant to be inoculated. A needle dipped in the juice contained in the mortar will now be used to prick the leaf about six or seven times along the midrib, using the label as a support. After dipping again, scratches are made along one side of the lamina parallel to the side veins, and after a further dip, along the other side of the lamina. Three leaves on each plant are usually so treated, and the end plant in the row of five is marked with a label bearing the date, the source of inoculum, and any other necessary information.

This method may be varied by introducing the juice to the leaves by means of a pipette and scratching with a

naked needle or a second wooden label. The square end of the latter could be dipped into the juice and used to 'paint' the leaf.

Tissue Mutilation. The inoculum-juice for this method is prepared in the same way as for needle inoculation, but more is required. The hands should first be washed with soap and water and then a piece of cheesecloth about 2 inches square is wrapped round the index finger of the right hand. After dipping in the extract, it is used to rub the surfaces of the leaves of plants to be inoculated, the index finger of the left hand or a wooden label being employed as a support for the leaf. Frequent dipping usually ensures success, for this is the most certain method for the transmission of a virus by 'mechanical' means. Rubbing breaks the hairs, and therefore provides numerous wounds through which the virus can enter. The plants are arranged and labelled as for needle inoculation.

Other Methods. A strong virus, such as that of ordinary Tobacco Mosaic, may sometimes be transferred to a healthy plant by enclosing the diseased extract on a leaf by means of a ring of petroleum jelly, or by scraping the midrib with a razor previously used to scrape a diseased plant. All these methods involve some mutilation, however slight, of the hairs of the healthy plant, and it is very doubtful whether a virus ever gains entrance to a healthy plant in the absence of a wound.

Stem Grafting. Any method of grafting or budding is capable of transmitting a virus across its union, but the method of cleft grafting is most generally useful for herbaceous plants, and will be here described.

The stock is usually the healthy plant; the scion bears the disease. It is highly advisable that some control on the continued health of the stock be obtained, and this may

be done by some method of vegetative propagation—the growing of a part of the same tuber as produced the stock in the case of the potato, or the striking of a cutting before grafting. The stock is prepared by cutting across at a node, and the severed part may be used as the 'control' cutting. A length of stem bearing buds is cut from the plant to form the scion, and a wedge-shaped piece, beginning just above a node and ending in another node, is formed by two clean cuts made with a flamed knife or safety-razor blade. The stem is then trimmed to include only one bud or the growing point. Any expanded leaves are trimmed down. A longitudinal slit is made in the stock, just shorter than the wedge of the scion, and the latter is then pressed firmly into the former. The joint is securely wrapped round with wet raffia. The grafted plant must be suitably labelled and must then be placed in a moist, shady position, as under the greenhouse bench, until the graft takes. In very dry greenhouses it may be necessary to remove the plants to a damp chamber after grafting, or a mass of absorbent cotton-wool may be wrapped round the union and kept moist. Symptoms of the disease will appear on the growth from the scion, and on the axillary shoots from the stock, if the graft has been successful.

When virus diseases of woody trees or shrubs are to be studied by means of grafting, the methods of crown grafting or whip-and-tongue grafting may be employed. These are described in any good text-book of gardening and will not be further dealt with here.

Tuber Grafting. This process is useful for the transmission of virus disease during the dormant period. It has hitherto been used mainly for potatoes, for which two methods have been devised—half-tuber grafting and core-grafting.

Half-tuber Grafting. The healthy tuber is cut in half

transversely by a flat cut made with a flamed knife, and the diseased tuber is similarly treated. The 'rose' half of the healthy tuber (which bears most of the buds) and the 'heel' or stolon half of the diseased organ are brought in contact along their cut surfaces. They are retained in position by means of a ligature of string or a rubber band. A little grafting wax applied round the junction is often beneficial. The diseased half is completely disbudded. A control on the health of the healthy tuber can be obtained by growing the heel end. The rose end of the diseased tuber provides a check on the source of disease.

Core-Grafting. The three largest members of a set of cork-borers ($\frac{1}{2}$–$\frac{5}{8}$ in.) are sterilized by flaming, and when cool the smallest is employed to take out a core (so arranged as to include an 'eye') from the healthy (stock) tuber. The core is removed to a covered dish lined with damp blotting-paper, where it will cork over and sprout. It may be grown as a control on the health of the stock tuber.

A borer one size larger (two sizes larger if the tuber is at all flaccid) is used to take out a core (which must not include an eye) from the diseased scion tuber. This core is immediately pushed into the hole in the stock tuber, and if one end protrudes, it may be trimmed level with a flamed knife. The diseased tuber with a hole (known as the 'source'), the graft-tuber, and the control core should all be suitably labelled with gardeners' ink.

This process of tuber grafting is best performed in early winter, the tubers being planted in spring.

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